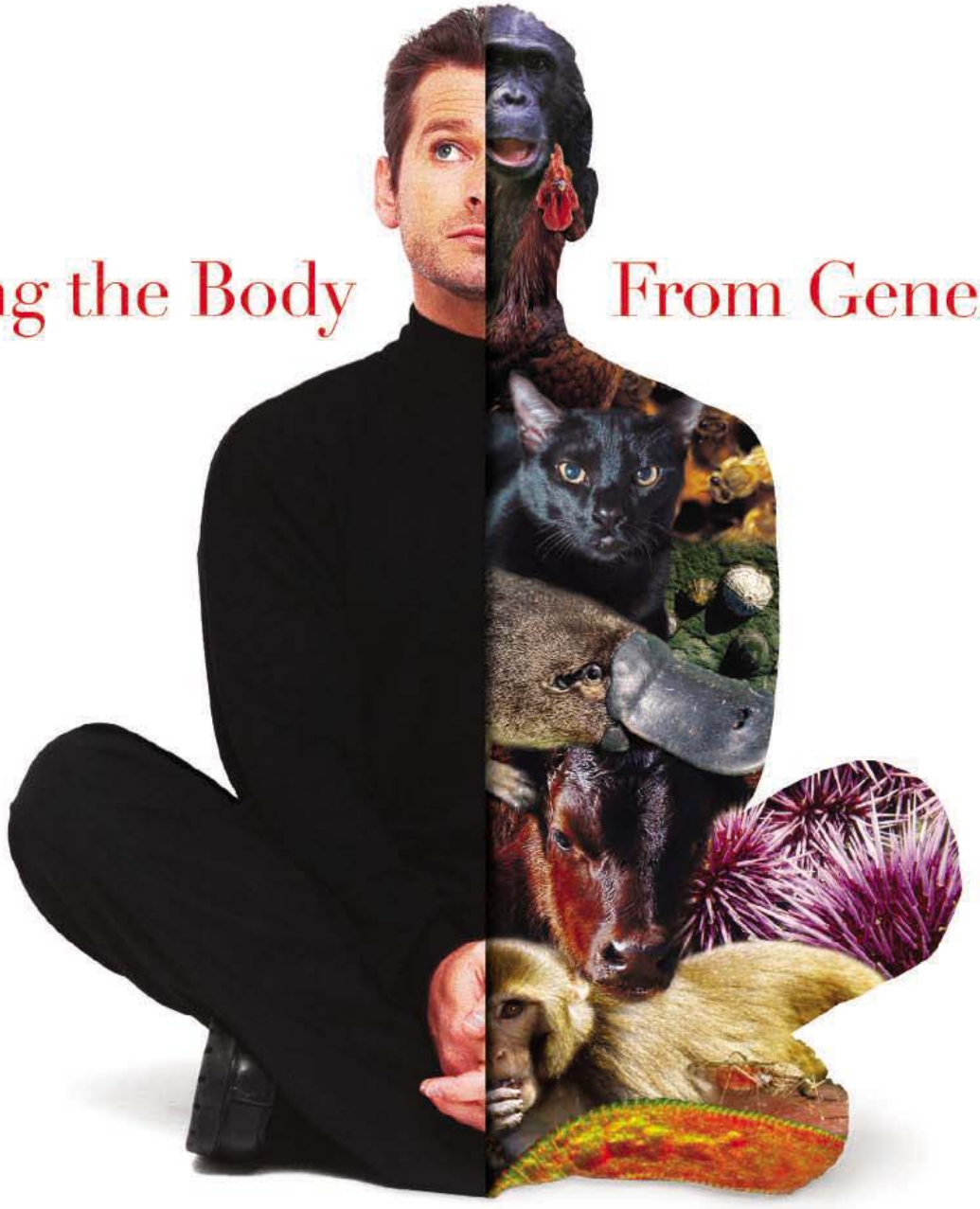
29 September 2006 | $10

Science

Building the Body From Genes



AAAS

Volume 313, Issue 5795

COVER

Through comparative genomics, scientists are learning about the forces that have promoted diversity or imposed constraints on our biological machinery. As described in a special section beginning on page 1907, comparative genomics not only gives us a view of our evolutionary history but also illuminates human physiology and suggests new approaches to attacking diseases.

Image: Kelly Buckheit Krause

>> Editorial p. 1853; Book Reviews pp. 1889 to 1892; Perspective p. 1897; Research Article p. 1929

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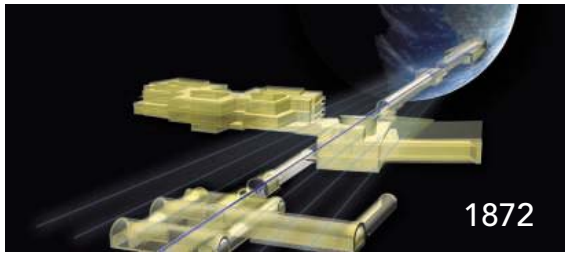

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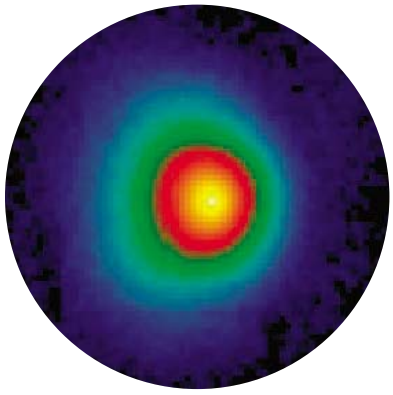

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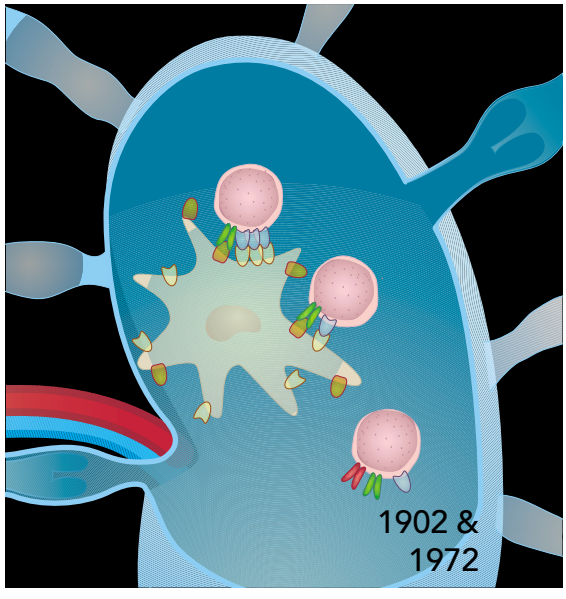

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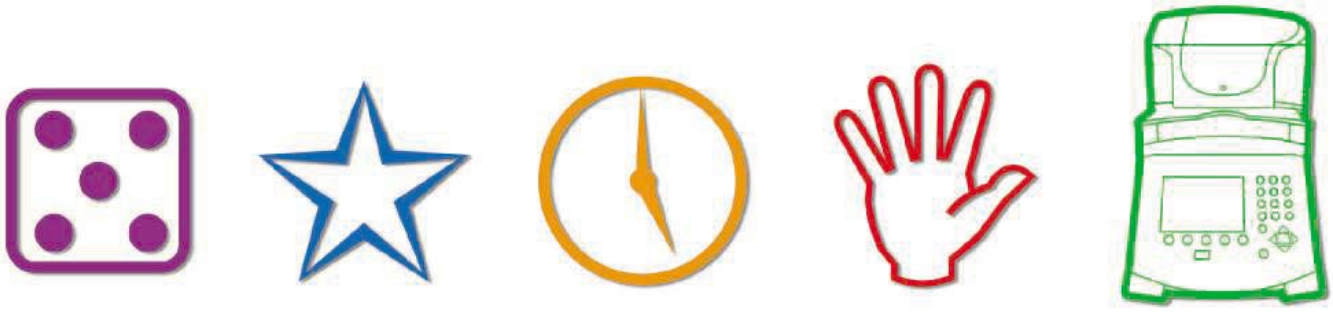

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Male Utah prairie dogs are more likely to be killed by predators when searching for a mate.

Adapting Itself Into Oblivion?
The black field cricket has evolved to avoid a predator, but now it's paying the price.

Classical's Class and Rap's Bad Rap
Many stereotypes about musical taste appear to hold true.

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SPECIAL SECTION

Genomic Evolution: Building the Body From Genes

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EDITORIAL GUIDE: Focus Issue—From Genes to Tissues
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Recent research implicates the MAPK pathway in promoting arterial fate.

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GLOBAL: Human Genetics and Health—An Overview of Career Opportunities
E. Pain
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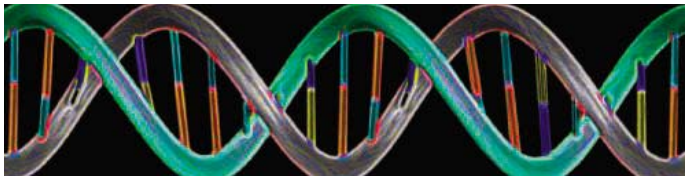

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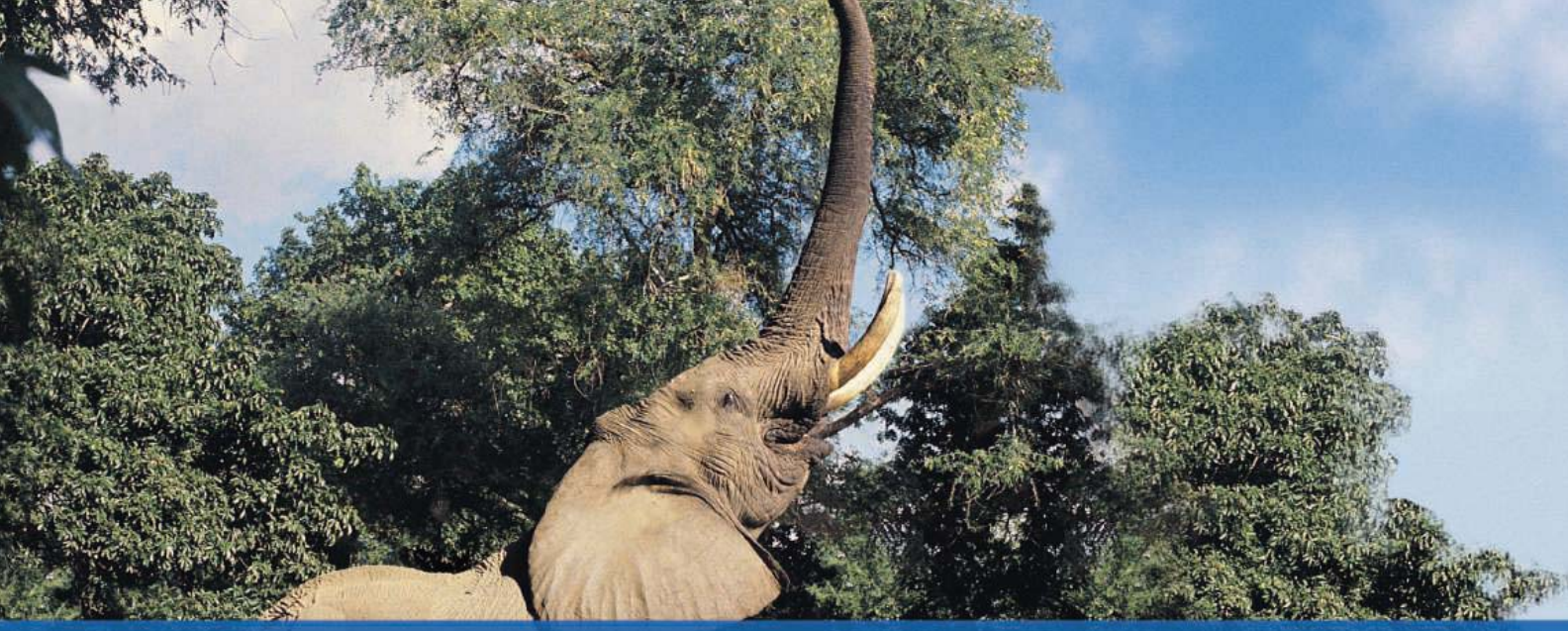

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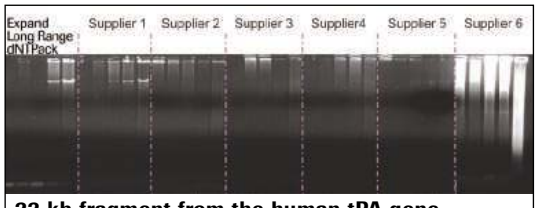
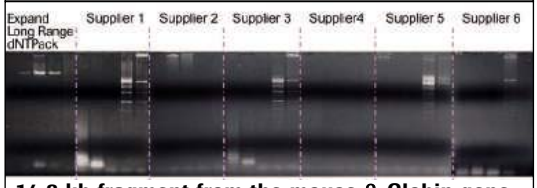


22 kb fragment from the human tPA gene



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EDITED BY STELLA HURTLEY AND PHIL SZUROMI

Sweet Smell of Communication

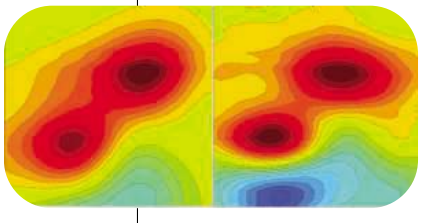
The aromas put out by plants serve to draw in insect pollinators, but they also enable communication with other plants. **Runyon *et al.*** (p. 1964; see the news story by **Pennisi**), studying a parasitic plant that is also a noxious weed, find that the dodder plant responds to volatile emissions from tomato plants such that the seedling parasite can rapidly locate and to latch onto a host plant. Wheat, which dodder generally disdains as a host, releases volatiles that include a seemingly repellent component. The function of volatile signals in this interaction between plants resembles the function of volatiles in signaling between insect herbivores and their plant fodder.

Field-Effect Modulation of Oxide Interfaces

Oxides tend to be insulators, but the interface region between two oxides can be grown to support a high-mobility, two-dimensional electron gas that can display a range of functional characteristics, such as superconductivity, magnetism, and ferroelectric behavior. Using oxide heterostructures, **Thiel *et al.*** (p. 1942, published online 24 August; see the Perspective by **Hwang**) now show the conductance of the interface region can be modulated over many orders of magnitude by applying an electric field. The versatility of these oxide materials and the ability to switch the behavior with an electric field bode well for potential applications.

A Lateral Look at Lipid Phases

Lateral heterogeneity in lipid bilayers can be difficult to assay at the length scale near 100 nanometers that has been associated with structures such as lipid rafts. Scanning probe methods provide sufficient spatial resolution but limited information on composition, and optical methods often have limited spatial resolution or introduce dye groups that may perturb the partitioning of lipid components. **Kraft *et al.*** (p. 1948; see the Perspective by **Groves**) have used a high-resolution, secondary-ion mass spectrometry probe and isotopic labeling to study supported bilayers of an equal mixture of DLPC (dilauroylphosphatidylcholine) and DSPC (distearoylphosphatidylcholine), which phase-separates at room temperature into a fluid phase and a gel phase. They identified variations in the gel-phase composition that may arise from small regions of trapped fluid phase.



CREDITS (TOP TO BOTTOM): JUSTIN RUNYON/COURTESY OF THE DE MORAES AND MESCHER LABS; ZHENG *ET AL.*

Tracking Down Mn(III)

Manganese, an important trace element in ocean biochemistry, is directly incorporated into enzymes and widely affects the chemistry in different sediment and water layers. Soluble Mn(III), an important intermediate species, has been thought to be absent in the environment, yielding Mn(II) and Mn(IV) species instead. **Trouwborst *et al.*** (p. 1955; see the Perspective by **Johnson**) have now documented the presence of Mn(III) in regions of the Black Sea and the Chesapeake Bay that are low in O_2. Ligands apparently stabilize Mn(III), which in turn stabilizes suboxic zones in all waters and water-rich sediments.

Clocking Spinning Carbons

Nuclear magnetic resonance (NMR) spectroscopy has long been used to measure hindered rotation rates about molecular single bonds, although its limitation to microsecond resolution has prompted chemists to categorize rotational barriers in terms of an "NMR time scale." **Zheng *et al.*** (p. 1951) show that an infrared vibrational analog to such NMR experiments can be used to clock the picosecond internal rotation dynamics of an ethane derivative at room temperature and shed light on the weak interactions that govern the low-energy isomerization barriers in such molecules.

Accelerated Melting

The Greenland Ice Sheet, the second largest ice sheet on Earth, is losing mass. **Chen *et al.*** (p. 1958, published online 10 August) report results from the Gravity Recovery and Climate Experiment (GRACE) satellite mission that indicate the Greenland Ice Sheet has been melting at an accelerated rate since 2004. It is now disappearing at the rate of about 240 cubic kilometers per year, which is three times as quickly as in the preceding 5 years. These results are consistent with other recent work that has used different techniques to estimate the mass balance of the ice sheet, and indicate that melting in Greenland is contributing enough water to raise global sea level by more than half a millimeter annually.

Mapping Biological Connectivity

Comprehensive catalogs of biological information (such as sequence or protein structure data) can have enormous utility in biomedical research. **Lamb *et al.*** (p. 1929) have extended this approach to create comprehensive catalogs of cellular states, as defined by RNA expression. The effects of 164 small molecules on the complete messenger RNA expression profiles were examined in established cell lines, with a primary focus on a breast cancer epithelial cell line. By comparing the genomic signature of drug candidates (the anticancer drug gedunin, estrogen, histone deacetylase, and phenothiazine antipsychotics) or a disease state (obesity, Alzheimer's disease, and dexamethasone-resistant acute lymphoblastic leukemia) to this resource, it was possible to identify potential mechanisms of action, confirm previous applications of known drugs, and identify additional potential uses for known drugs.

Continued on page 1851

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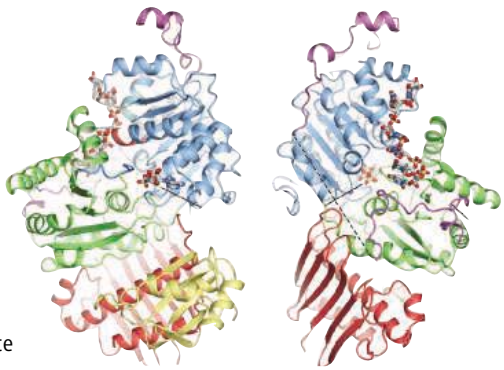
Ribosome Structure at Higher Resolution

Significant insights into the mechanism of protein translation have come from recent high-resolution structures of the 50*S* and 30*S* ribosomal subunits. Progress has also been made on determining the structure of the whole ribosome, but a high-resolution view of the entire ribosome bound to its ligands has been lacking. **Selmer *et al.*** (p. 1935, published online 7 September) have determined the structure of the *Thermus thermophilis* ribosome complexed with messenger RNA (mRNA) and transfer RNA (tRNA) at 2.8 angstrom resolution. The structure reveals details of the interaction of the mRNA and tRNA ligands with the ribosome and the role of proteins and metal ions in the formation of intersubunit bridges.

Immune Cells and Cancer Prognosis

In the mouse, the immune system can recognize a developing tumor and control its growth, but whether the same is true in humans has been controversial. To investigate the impact of the immune response on the prognosis of cancer patients, **Galon *et al.*** (p. 1960; see the news story by **Couzin**) analyzed tumor-infiltrating immune cells in human colorectal cancers by gene expression profiling and in situ immunohistochemistry. In three independent patient populations, the properties of the immune cells (type, density, and location) within the tumors were a better predictor of recurrence and overall patient survival than tumor histopathology. Thus, information about the immune response in individual cancer patients could help optimize treatment decisions.

Exon Junction Complex Revealed



Exon junction complex (EJC) assembles on newly spliced RNA and is a central effector of messenger RNA functions. **Andersen *et al.*** (p. 1968, published online 24 August) have determined a 2.3 angstrom resolution structure of a core EJC complex bound to an RNA oligonucleotide. The EJC core comprises the DEAD-box RNA helicase eIF4AIII bound to an adenosine triphosphate (ATP) analog, and three additional proteins—MLN51 , MAGOH, and Y14. Tight binding of the complex to RNA requires that ATP hydrolysis by eIF4AIII is inhibited. The structure shows how eIF4AIII binds sequence-independently to the RNA backbone and how the protein partners participate in RNA recognition and regulate ATP hydrolysis of the DEAD-box helicase.

CREDIT: ANDERSEN *ET AL.*

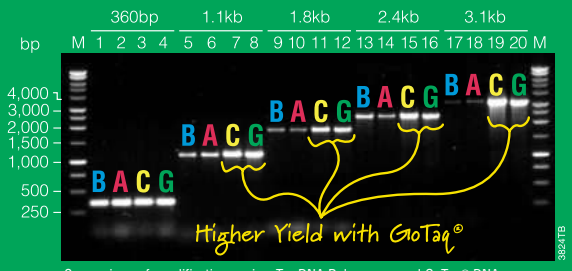
Stop to Start

The T cell surface receptor CTLA-4 helps dampening immune responses, and deficiency in the protein can lead to uncontrolled immune activation and autoimmunity. This effect has been attributed to the loss of negative signals that down-regulate T cell activation. **Schneider *et al.*** (p. 1972, published online 24 August; see the Perspective by **Mustelin**) tracked T cells as they interacted with activating dendritic cells in culture and in vivo. CTLA-4 appeared to stimulate roaming of T cells away from dendritic cells, which lessened the likelihood that the T cells would remain activated. This finding makes CTLA-4 a potentially important clinical target.

Muscle Building

When neurons innervate muscles, they secrete a protein, agrin, which causes neurotransmitter receptors to cluster on the muscle and form of a synapse at the point of nerve contact. A muscle-specific kinase is necessary for synapse development, as is the recently described protein Dok-7. Congenital myasthenic syndromes (CMS) are a group of inherited disorders of neuromuscular transmission, which lead to muscle weakness. **Beeson *et al.*** (p. 1975, published online 17 August) now find that a group of patients with CMS have mutations in Dok-7. These mutations result in the formation of small, abnormal synapses at the neuromuscular junction and help account for the symptoms of the disease.

Astonishingly robust. Want to try some?



Comparison of amplifications using *Taq* DNA Polymerase and GoTaq® DNA Polymerase. B; *Taq* DNA Polymerase in Storage Buffer B. A; *Taq* DNA Polymerase in Storage Buffer A. C; GoTaq® DNA Polymerase in Colorless GoTaq® Reaction Buffer. G; GoTaq® DNA Polymerase in Green GoTaq® Reaction Buffer.

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Kathy L. Hudson is director of the Genetics and Public Policy Center in Washington, DC. She is former assistant director of the National Human Genome Research Institute at the National Institutes of Health. E-mail: khudson5@jhu.edu

Genetic Testing Oversight

A MAJOR IMPACT OF SEQUENCING THE HUMAN GENOME HAS BEEN THE ABILITY TO DETECT disease and the risk of disease through genetic testing. Today, there are genetic tests for more than 1000 diseases, and that number is increasing rapidly. Given the potential powerful health consequences of genetic test results, shouldn't someone be in charge of making sure that the tests are accurate and reliable? Amazingly, in the United States no one seems to be, despite a direct congressional mandate and a very clear public expectation that there be such oversight. How has such a failure come about, and what should be done to remedy the situation?

During the 1990s, in anticipation of the "genetic revolution" in medicine, numerous government and other advisory bodies recognized that the rules governing garden-variety laboratory tests were simply insufficient for the age of new genetics. They recommended that the Centers for Medicare and Medicaid Services (CMS), the agency within the U.S. Department of Health and Human Services (DHHS) that is responsible for the quality of clinical laboratories, beef up the standards for genetic testing laboratories. CMS was charged with adopting new regulations to guide a smooth translation of genetic testing from research to practice. Key among these recommendations was explicit enhancement of the accuracy and reliability of genetic testing under the Clinical Laboratory Improvement Amendments of 1988 (CLIA).

One would expect, then, that CMS has been active in ensuring that genetic testing laboratories are getting it right. After all, proficiency testing is mandatory for labs that perform diagnostic tests in microbiology, immunology, and clinical chemistry. In 2000, CMS announced that it would develop such tailored regulations for genetics. But nothing has happened for the past 6 years, leaving a system in place that still does not routinely evaluate the competence of genetic testing labs.

Things did look up when, in April 2006, DHHS placed the creation of genetic testing rules on its regulatory agenda, with a target date of November 2006. This announcement was received enthusiastically by diverse patient advocacy groups, health care provider organizations, industry, and genetic testing laboratories, which collectively urged expeditious action. Three months later, inexplicably, the government abruptly reversed course. CMS now asserts that creating regulations to ensure the quality of genetic testing laboratories lacks sufficient "criticality" to warrant rulemaking, and that existing CLIA regulations are adequate to protect the public health.

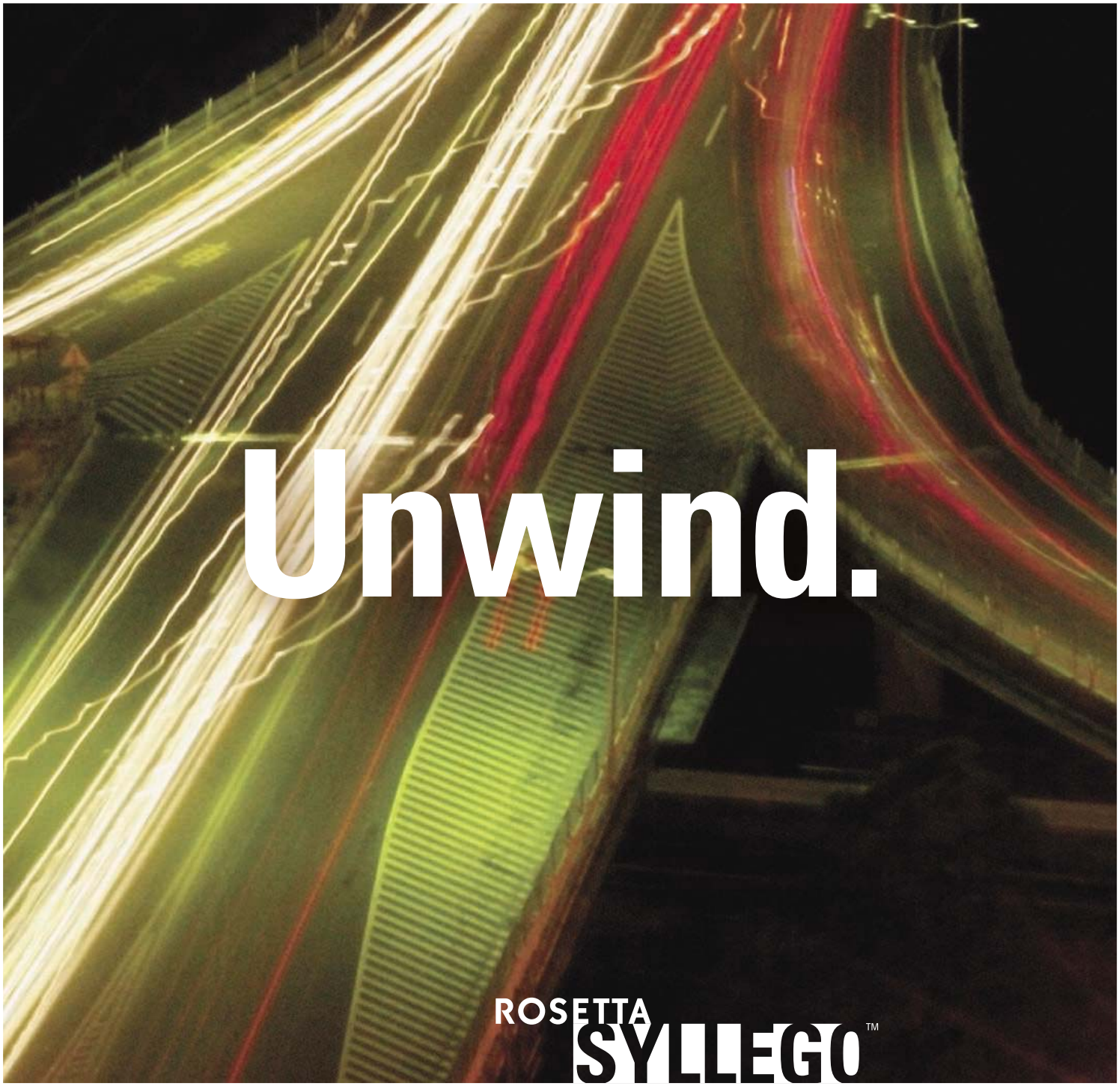
Existing regulations? A Senate hearing in July 2006 released a Government Accountability Office report that detailed fraudulent genetic tests offered over the Internet, and the failure of one of the laboratories doing the testing to deliver consistent results using the same DNA. A 2006 survey by the Genetics and Public Policy Center found that, in the United States, at least a third of genetic testing labs fail to perform proficiency assessments for some or all of their tests, and that analytic errors increase in direct proportion to the failure to perform proficiency testing. Draft guidelines for genetic testing quality released in 2006 by the international Organization for Economic Cooperation and Development similarly identified proficiency testing and lab quality as key to ensuring public health worldwide.

We know intuitively and empirically that errors in genetic testing can have tragic consequences. We need to forge and enforce rules—the right rules—to ensure the quality of genetic testing. Laboratories should be required to demonstrate that they can reliably perform the tests that they sell. And when they do poorly on proficiency testing, health care providers and the public have the right to know so that they can make wise health care decisions. That responsibility sits squarely with CMS. The Food and Drug Administration's (FDA's) jurisdiction also bears on genetic testing, and this year it has shown vigor in drafting guidelines for the safety of certain genetic tests. But the FDA's efforts cannot substitute for CMS doing its job to ensure laboratory quality.

At worst, genetic testing errors can kill; at best, they result in poorly spent health care dollars. Moreover, should the public begin to question the accuracy of genetic tests or insurers begin to question their validity, "personalized medicine" will be nothing more than a postscript on the pages of medical history. We need sensible regulation to secure the future of genetic medicine.

– Kathy L. Hudson

10.1126/science.1134996



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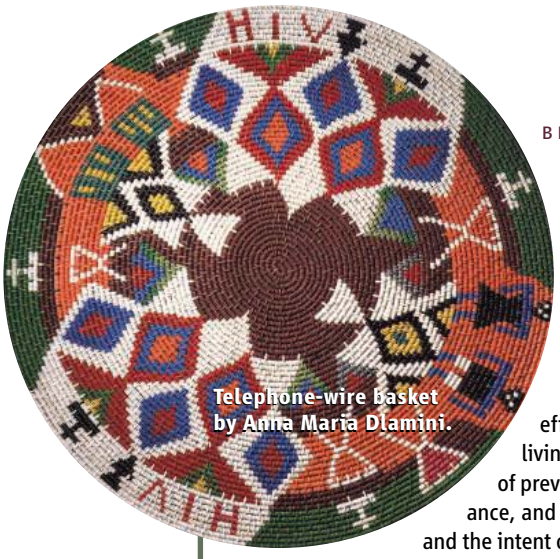


Telephone-wire basket by Anna Maria Dlamini.

BIOMEDICINE

To Have or Have Not

The rollout of antiretroviral therapy during 2004–2008 in South Africa brings with it severe ethical dilemmas regarding the allocation of drugs, because supply will outstrip need. Incorporating data from KwaZulu-Natal in a spatially explicit mathematical model, Wilson *et al.* have applied the government's allocation strategy in three scenarios to predict the consequences of different choices.

The modeling reveals clearly that for preventing transmission, the most effective strategy is to concentrate all of the doses (for half a million people living with HIV) in Durban, where HIV prevalence is 13%. This will have the effect of preventing 15,000 infections by 2008, minimizing the transmission of drug resistance, and preventing the greatest number of deaths. But this choice is not egalitarian, and the intent of antiretroviral therapy is treatment and not prevention. However, if the drug allocation were split between urban and rural areas (just over half of the KwaZulu-Natal population is rural, with an HIV prevalence of 9%), its effectiveness for HIV prevention would be reduced by about a third to a half, not just because the rural population is dispersed but also because the rural health infrastructure is relatively weak and because distribution and clinical monitoring will not be so effective. — CA

Proc. Natl. Acad. Sci. U.S.A. **103**, 14228 (2006)

PSYCHOLOGY

Saving Face

It is well established that the perception of probabilities can be influenced by how a particular likelihood is framed or anchored, and that the consequences for a patient if the number qualifies a medical prognosis can be real and serious. Bonnefon and Villejoubert propose another context in which the diagnosis of a possible condition is not perceived to reflect its likelihood but instead is taken as warning of a dire outcome. Upon quizzing subjects (recruited by and not representative of psychology students) after a physician had delivered an assessment of possible insomnia or deafness, they found that the condition regarded as more serious (deafness) was judged to be more likely to occur and that the use of the word possible was interpreted as a means of softening the news. In contrast, subjects who adhered to a probabilistic interpretation of the phrasing believed that both conditions were equally likely outcomes, underlining the importance of mutual understanding in physician-patient discussions. — GJC

Psychol. Sci. **17**, 747 (2006).

CHEMISTRY

Twisting with Fewer Breaks

In polymer synthesis, chiral solvents can be used to increase the stereoregularity, or tacticity, of polymer chains. However, in the synthesis of some polymers, the alternation between helical senses also affects the distribution of chain lengths. Holder *et al.* point out that in the Wurtz-type reductive coupling of dichloro-diorganosilanes to form polymers on supported sodium metal, regions of the chain where the helical sense reverses (switching from *P* to *M* screw sense) are prone to backbiting reactions that terminate chain growth. The authors succeeded in raising the proportion of longer chains in the product distribution by running the reaction in enantiomerically pure limonene, a relatively inexpensive and unreactive chiral liquid. This effect increased at higher reaction temperatures: At 90°C, the weight-average molecular weight, measured using size exclusion chromatography, more than doubled when the optically pure solvent was used in place of racemic limonene, presumably because of a reduced number of reversal sites. Investigation by optical absorption and circular dichroism spectroscopy supported a mechanism in which the chiral solvent stabilized a particular helical conformation of the growing polymer chain. — PDS

J. Am. Chem. Soc. **128**, 10.1021/ja064587e (2006).

ECOLOGY/EVOLUTION

Small-World Networks

Loss of habitat is a pervasive problem affecting species and ecological communities, yet our ability to predict the effects of habitat loss on the population sizes of the species is surprisingly limited; this can, in turn, be a hindrance to conservation planning. However, there is increasing correlative evidence that the structure of food webs might provide important clues to the patterns of population change, and Gotelli and Ellison confirm this with experimental evidence from invertebrate communities inhabiting *Sarracenia* pitcher plants. The experiments involved reducing the volume of water in the pitchers and removing the predators at the top of the food chain (which are often the first casualty of habitat loss), and then monitoring the changes in abundance of the remaining species. The observed patterns of population change most closely conformed to path analytical models that incorporated food web structure. Despite the small size and relative simplicity of the *Sarracenia* microecosystem, the structure of its food web is similar to that of larger ecosystems. Hence, it is plausible that these models can be used to predict patterns of abundance in response to habitat loss at larger scales. — AMS

The pitcher plant.

PLoS Biol. **4**, 10.1371/journal.pbio.0040324 (2006).

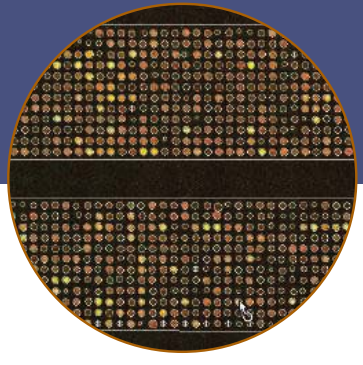
GEOPHYSICS

Westward Migration

The effects of Hurricane Katrina have been felt further afield seismically as well as politically. Gerstoft *et al.* detected seismic activity in Califor-

Continued on page 1857

More Microarray Research



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Continued from page 1855

nia corresponding to pressure and surface waves generated by pounding ocean waves in the Gulf of Mexico during the height of the storm, 28 to 29 August 2005. The seismologists used beam-forming techniques to back-project very low frequency seismic energy received at an array of stations in southern California. Body waves at double the ocean wave frequency (0.1 to 0.2 Hz) rattled deep through the earth from their source in shallow water east of New Orleans during the storm and for 9 hours after its landfall. Surface waves were also detected across the Gulf and tracked the ocean wave frequency and higher harmonics. The seismic surface waves mimicked the ocean wave pattern, with higher frequencies emanating from the eastern side and lower frequencies to the west of the eye. Thus, both surface and body seismic waves were generated in shallow water by breaking ocean waves from Katrina, but different physical mechanisms couple the water and ground motions that produce them. — JB

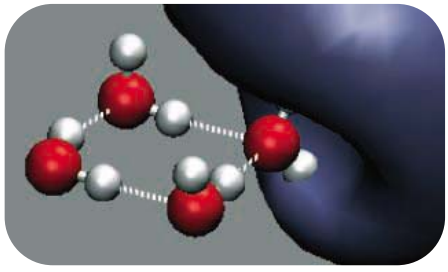
Geophys. Res. Lett. **33**, 10.1029/2006GL027270 (2006).

CHEMISTRY

Wet Quanta

Modern computers routinely allow efficient calculation of the geometries and electronic structures of neutral molecules using quantum-mechanical principles. However, charged species present a considerably greater challenge, and excess electrons are often treated by means of classical approximations. Herbert and Head-Gordon describe a method whereby the spatial distribution and detachment energy of an excess electron bound to a tetrameric water cluster can be computed quantum-mechanically. These hydrated water clusters have been the subject of extensive recent experimental study in light of the fundamental questions they raise about bonding motifs, as well as their role as models for bulk hydrated electrons of interest in biological electron transfer and photodamage.

To render the method computationally tractable, the authors propagate the cluster atoms along a classical trajectory while applying



Calculated spatial distribution (blue) of an excess electron in a water cluster.

ab initio Møller-Plesset perturbation theory at each step to solve the electronic structure. The simulation results agree well with recent experimental measurements of vibrational and photoelectron spectra, and furthermore allow estimation of the cluster temperatures based on observed spectral widths. The authors note in closing that further advances in computing power should extend the applicability of the method to larger molecular clusters. — JSY

Proc. Natl. Acad. Sci. U.S.A. **103**, 10.1073/pnas.0603679103 (2006).

CREDIT: HERBERT AND HEAD-GORDON, *PROC. NATL. ACAD. SCI. U.S.A.* **103**, 10.1073/PNAS.0603679103 (2006)

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<< How Nickel Binding Regulates Transcription

Bacteria, such as *Escherichia coli*, have enzymes that require nickel ions, and they express a nickel transporter to acquire the metal from the environment. To keep cellular concentrations of nickel relatively constant, the expression of the transporter is regulated by a repressor, NikR, which is itself a nickel sensor. NikR binds to the operator of the transporter gene and represses transcription only when it is in the nickel-bound form. Schreiter *et al* solved the crystal structures of the nickel-bound form of NikR from *E. coli* both alone and in a complex with a DNA fragment corresponding to the promoter of the nickel transporter gene. The protein has two DNA-binding domains that interact with sites in the palindromic operator on either side of a metal-binding domain. In other ligand-regulated transcription factors, activation is proposed to occur when a change in the spacing between the DNA-binding domains is altered in such a way that they interact more effectively with the promoter DNA. Comparison of the new structures with the previously reported structure of nickel-free protein indicates that this is not how NikR works. Rather, it appears to create a new interactive surface within the metal-binding domain that enhances the interaction of the protein with the promoter DNA helix. The results provide a detailed look at the precise molecular changes that underlie transcriptional control by a ligand-regulated transcription factor. — LBR

Proc. Natl. Acad. Sci. U.S.A. **103**, 13676 (2006).

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See pages 102 and 103 of the 6 January 2006 issue or access www.sciencemag.org/feature/contribinfo/home.shtml

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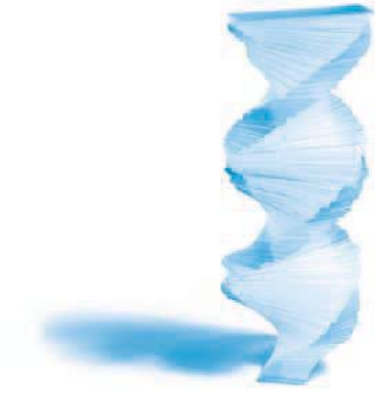

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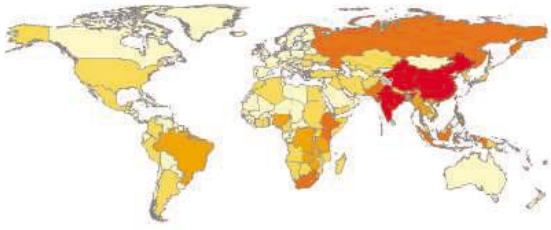
DATABASE

The Planet's Medical Chart

If you want to find out which countries recorded the most cholera cases last year or compare measles vaccination rates, drop by the Global Health Atlas from the World Health Organization. The site emphasizes communicable diseases, but its cache of health statistics covers variables as diverse as child mortality and number of hospital beds per capita. Last year, for instance, Senegal reported the most cholera cases, nearly 32,000. And Bahrain ranks highest in measles vaccination levels at 100%—versus 93% in the United States and only 80% in the United Kingdom. A library houses a host of maps, or you can use the site's data to make your own charts. Right, a breakdown of world tuberculosis cases in 2004, with red indicating the countries with the most infections. >> globalatlas.who.int/globalatlas



AUDIO

Tuning In Biomedical Research

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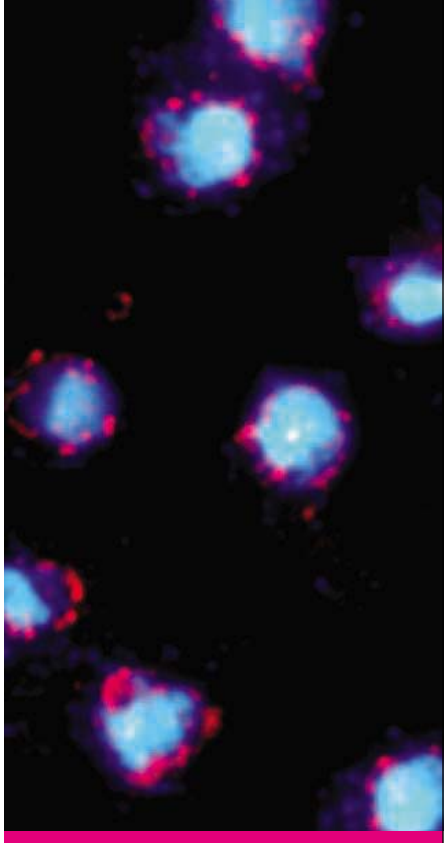

DATABASE

PROTEINS AT HOME

The protein Nsp1 forms part of the nuclear pore, the channel that passes through a cell's nuclear membrane. The molecule (pink in the image above) usually hangs out at the rim of the nucleus (blue). Track down Nsp1 and more than 30,000 other proteins with Organelle DB, started by molecular biologist Anuj Kumar of the University of Michigan, Ann Arbor. You can search protein localization data for 138 species, including lab stalwarts such as *Drosophila* and *Caenorhabditis elegans* and more exotic creatures such as the pygmy chimp *Pan paniscus*. Using information from the Kumar lab's experiments, the literature, and other databases, the site narrows each protein's whereabouts among more than 50 organelles and other cellular locales. Launch the new Organelle View feature to map your favorite yeast proteins on a three-dimensional cell model. >> organelledb.lsi.umich.edu

EXHIBIT

<< Life in the Volcano

The searing, acidic waters of the Uzon Caldera on Russia's Kamchatka Peninsula are paradise for microorganisms like these colorful bacteria and algae (left). At this new exhibit from the Exploratorium in San Francisco, visitors can tag along with U.S. and Russian researchers who choppered into the remote collapsed volcano last year. The scientists narrate slideshows about their work on extremophiles. One group, for instance, is studying traces that modern bugs leave in the minerals that precipitate around hot springs. They hope to find ways to more easily identify microbial remains in ancient rocks—and possibly in extraterrestrial samples. >> www.exploratorium.edu/kamchatka

DICTIONARY

Physics Law School

Unlike Rome at rush hour, the universe is a lawful place. Rules govern everything from the relation between a gas's pressure and volume to the speed at which galaxies recede from Earth. Catch up on physics jurisprudence with The Laws List from Erik Max Francis, a programmer in San Jose, California. For example, Lambert's first law relates the amount of illumination falling on a surface to its distance from the light source. The site also serves as a physics glossary, offering brief explanations of terms and ideas. >> www.alcyone.com/max/physics/laws

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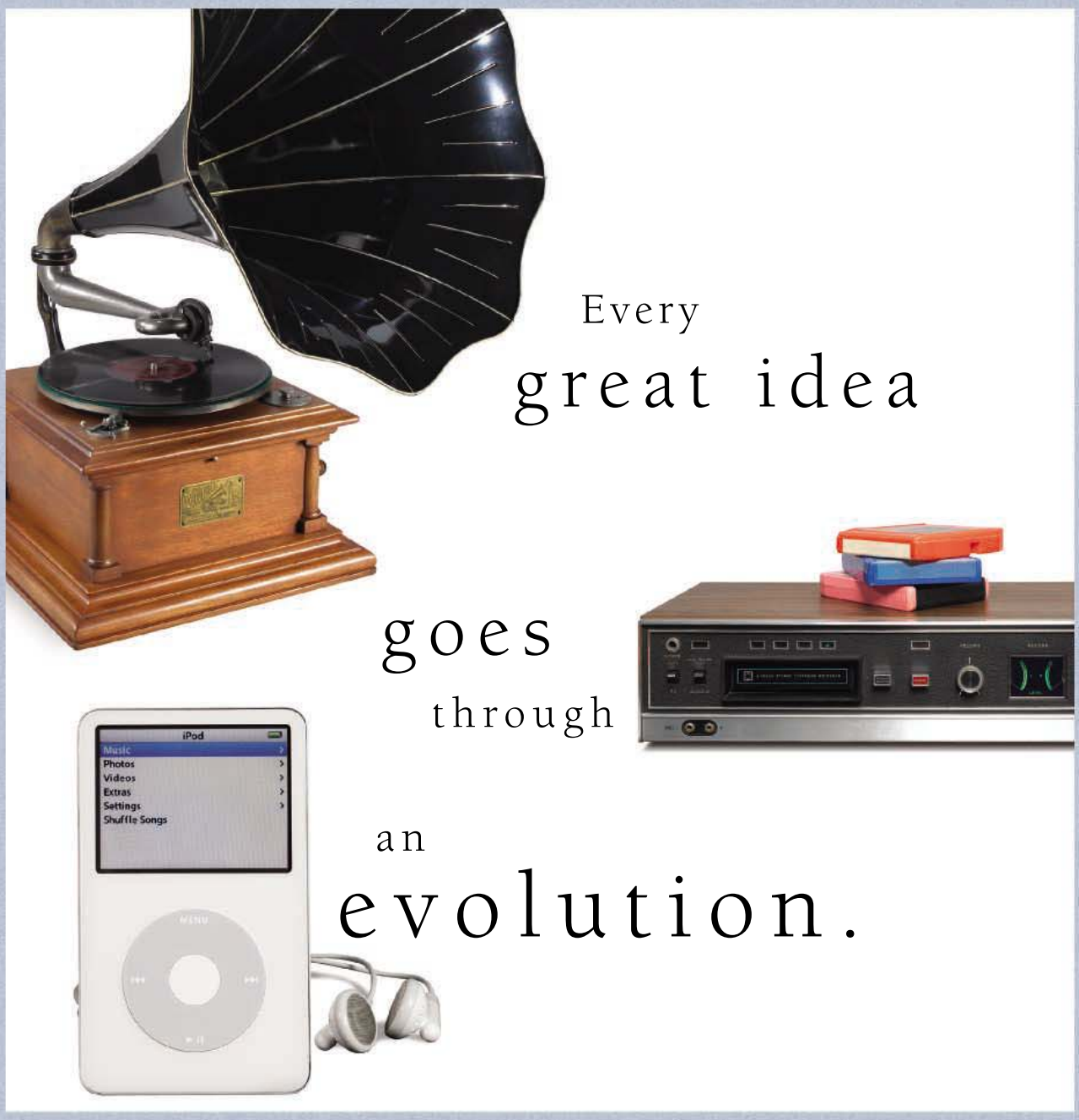

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Jarawa child.

<< Outsiders Threaten Stone Age People

Only two relatively isolated indigenous tribes remain in the Andaman Islands in the Bay of Bengal (*Science*, 7 July, p. 34). Now an Indian government report says that one group, the Jarawa, is being threatened by increasing contact with outsiders.

There are only 306 Jarawa hunter-gatherers who live on a 1028-km^2 reserve. Geneticists are interested in them because they are closely related to the humans who first migrated to the islands 60,000 years ago (*Science*, 13 May 2005, p. 996). But the report says construction of a road into the Jarawa territory, and the outsiders it is bringing in, are having a "devastating" impact. It says sexual exploitation of girls by police and roadworkers is threatening both the health and the genetic integrity of the Jarawa, who have so far avoided contracting modern diseases. It also points to recent studies by Lalji Singh and colleagues at the Centre for Cellular and Molecular Biology in Hyderabad, India, that indicate the Jarawa are unusually susceptible to the AIDS virus.

The report recommends the creation of a Jarawa Tribal Development Authority that might help limit outside contact with the tribe. Survival International, a London-based group that works for the rights of indigenous communities, has submitted a petition signed by 50,000 people asking the government to close the controversial road.

Egyptian Collection Finds Home at Last

The Petrie Museum, one of the world's biggest collections of ancient Egyptian artifacts, has been "temporarily" housed at University College London (UCL) since 1953. But its 80,000 objects are finally going to have a permanent home. Groundbreaking began this month on a new building on UCL grounds, to open in 2010.

The museum is named for Egyptologist William Flinders Petrie (1853–1942), whose excavations provided a wealth of objects from daily life such as pottery, lamps, and jewelry ranging from prehistoric times to the Islamic period. The $53 million project is good news to the archaeologists who now flock to the Petrie's cramped quarters to do research. Andreas Effland of the University of Hamburg in Germany says that the collection is "really fantastic" because it allowed him to fit together fragments of artifacts unearthed during recent German excavations at Abydos with pieces that Petrie found 100 years ago. Photographs from the collection are at www.petrie.ucl.ac.uk/index2.html.

Ancient Egyptian mousetrap.

CREDITS (TOP TO BOTTOM): AP; MSB; PETRIE MUSEUM

Dieting Mice Out of Style?

To the dismay of some longevity researchers, the National Institute on Aging (NIA) wants to stop supplying them with hungry old rodents. Citing waning demand and costs amounting to $800,000 a year, NIA announced this month that by 2013 it may close its long-running colony of mice and rats raised on a calorie-restricted diet.

Rodent studies have shown that restricting calories can extend life span. "It's a real blow to development of knowledge in this area," says Roger McCarter of Pennsylvania State University in State College, a former president of the American Aging Association. On the other hand, Roger McDonald of the University of California, Davis, says he and others now raise their own low-cal colonies in order to have more control over conditions.

Orders for NIA's calorie-restricted rats dropped by half between 2001 and 2005, to 481, says Nancy Nadon, who oversees the colony. Ironically, due to "soar[ing]" demand, NIA says it is running short of elderly non-calorie-restricted rodents, of which it disburses about 30,000 a year.

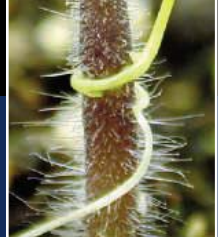
SEEDS AWAKENED FROM BIG SLEEP

Scientists at the Millennium Seed Bank (MSB) in West Sussex, U.K., have coaxed sprouts from South African seeds that have lain dormant for 2 centuries.

The seeds were collected by a Dutch merchant en route home from China in 1803. He stored 40 small packets in a notebook, which was taken when the British navy seized his vessel. The seeds, from 32 species, were rediscovered this year in the National Archives in London by a visiting Dutch researcher.

MSB researchers cut the seeds' hard outer shells and, because wildfires followed by rain are an important natural growth trigger, they soaked the seeds in water through which they had bubbled smoke. Most were duds, but bright green shoots erupted from three of them, the scientists announced last week. MSB ecologist Matthew Daws says DNA sequencing will help scientists locate mutations that may have spread through plant populations in South Africa over the past 200 years.

Although plants have been grown from 1200-year-old sacred lotus seeds, biologist Jane Shen-Miller of the University of California, Los Angeles, says "very few other seeds are known to remain viable for more than a few decades." Studying such time capsules, she adds, could reveal how seeds "overcome and repair cellular damage" incurred during their long hibernation.



A plant sniffs out its victim 1867

Stem cells from nondividing embryos 1869

GENETICS

Pollen Contamination May Explain Controversial Inheritance

Eighteen months ago, plant researchers shook the foundations of genetics with a gene that seemed to defy the basic rules of inheritance. *Arabidopsis* plants carrying only mutant versions of the gene, called *HOTHEAD*, somehow gave rise to progeny carrying a wild-type allele supposedly missing from both parents. These startling results led Susan Lolle and Robert Pruitt, plant geneticists at Purdue University in West Lafayette, Indiana, and their colleagues to propose that the plant's cells carried a hidden stash of genomic information, with the memory of the wild-type gene sequence encoded in RNA. Every once in a while, that RNA would correct the mutated *HOTHEAD*, they suggested.

The work, reported in the 25 March 2005 issue of *Nature*, stunned plant biologists, who have since offered several explanations for how the mutant gene could be corrected; one graduate student's proposal on his blog even became part of a *Plant Cell* paper.

But has the whole episode been much ado about nothing? Yesterday, in an online letter published by *Nature*, Steve Jacobsen, a Howard Hughes Medical Institute investigator and plant geneticist at the University of California, Los Angeles (UCLA), and several colleagues argued that undetected contamination—wild-type pollen that accidentally fertilized plants considered to be self-fertilizing—may have "reintroduced" old versions of the *HOTHEAD* gene. "Contamination has always been the simplest explanation," says Luca Comai, a plant geneticist at the University of California, Davis. "I would bet 100 against 1 that it's all explained by pollen contamination."

Lolle and Pruitt are willing to play those odds, noting that they conducted tests to rule out such contamination. "We've spent a lot of time trying to put the nail in the coffin of the outcrossing problem," says Lolle.

When Lolle, now at the University of Waterloo in Ontario, Canada, Pruitt, and their colleagues originally bred homozygous mutants of *HOTHEAD*, they found that 10% of the progeny wound up with one corrected *HOTHEAD* sequence, and in two cases, both mutant alleles were fixed. Moreover, when the researchers fertilized wild-type plants with pollen from a homozygous *HOTHEAD* mutant, 8 of 164 resulting seedlings had two wild-type alleles; all should have been heterozygous.

Stuck. Instead of opening (*left*) to allow pollen-laden stamen access to the female stigma, fused mutant flowers (*right*) trap pollen, hindering self-fertilization.

Arabidopsis typically reproduces through self-fertilization, but the researchers checked for contamination from other plants. Unpublished DNA fingerprint experiments, they say, pinned down which plant's pollen sired progeny with the restored wild-type allele. "Based on those fingerprints, stray pollen doesn't account for the changes we've seen," Lolle says.

Originally thrilled by the mutant *HOTHEAD* results, Jacobsen and postdoctoral fellow Peng set up an experiment to monitor restoration in it and another mutated gene. They found that if one of the genes was corrected, so was the other, suggesting an outside source of the wild-type alleles. The researchers then grew some *HOTHEAD* mutants in a room with a mix of other strains with known genotypes, while at the same time growing other *HOTHEAD* mutants in isolation.

For the latter plants, they took extra precautions. To avoid chance contamination from pollen in the air, on an insect, or on the person tending the plants, Jacobsen took some plants home and asked his wife to water them. Peng even sent seeds home to his parents in China. And a UCLA mathematician and a computer scientist were recruited to set up their own nurseries. "We wanted to make sure these were people who had no contact with *Arabidopsis*," says Jacobsen.

In mixed company, the wild-type *HOTHEAD* regularly reappeared, replicating Lolle and Pruitt's results. For one particular mutant, the wild-type *HOTHEAD* made a comeback in 156 out of its 994 progeny. But some of those 156 contained genes, such as one for a green-fluorescing protein, that could only have come from one of the plants nearby. And in 2735 cases when a plant with mutant *HOTHEAD* was grown in isolation, no wild-type version of the gene showed up. "When Jacobsen took great pains to isolate the plants, he couldn't reproduce the [reversion] phenomenon," notes Steven Henikoff, a geneticist at the Fred Hutchinson Cancer Research Center in Seattle, Washington.

The *HOTHEAD* mutation itself may be to blame for all the confusion. In the mutant plants, the sepals don't open, keeping the stamens—and their pollen—penned in, while the stigma protrudes out, ready to receive any stray pollen. "We believe that would decrease the efficiency of self-pollination" and promote cross-fertilization, says Jacobsen.

Pruitt and Lolle aren't sure what to make of these new data. "I can't explain his results just as he can't explain mine," says Pruitt. But he does concede that although their plants grew in plastic sleeves, another plant's pollen could have gotten in from outside. "Ours were not in as complete isolation as Steve's," says Pruitt. "I want to repeat Steve's experiments and see what we can see." Until that happens, the jury is still out about whether *HOTHEAD* is a true genetic outlaw. **–ELIZABETH PENNISI**

CREDIT: STEVE JACOBSEN/HHMI

GEOLOGY

Mud Eruption Threatens Villagers in Java

As a torrent of hot mud swamped rice paddies and inundated villages on the Indonesian island of Java, emergency crews last week started drilling a well to plug the eruption. A gas exploration project appears to have punctured a 2700-meter-deep geologic formation, releasing an unprecedented volume of pressurized steam and water that is carrying a river of mud to the surface. The geyser, which began in May, has made more than 10,000 people homeless and put many out of work as well. Experts say it may take until late November to shut down the leak.

The accident occurred near the coastal city of Sidoarjo, about 700 kilometers east of Jakarta, reportedly while the firm Lapindo Brantas Inc. was drilling an exploratory gas well. According to Rudi Rubiandini, a petroleum engineer at Institut Teknologi Bandung and adviser to Indonesia's Ministry of Environment, drilling had reached a depth of about 2800 meters when the accident occurred on 29 May. The drill string had become stuck in the well, he says, and while the crew was trying to free it, a geyser of mud and water erupted from the ground about 150 meters away.

Rubiandini says the well goes through a thick clay seam from 500 to 1300 meters, then sands, shales, volcanic debris, and into permeable carbonate rock. Highly pressurized hot water and steam from the carbonate formation, he says, appear to have broken out below the point where the equipment was stuck in the well and either eroded a channel to the surface or followed natural fractures. Along the way, the river of hot, brackish water is eroding the clay layer to brew the hot mud that eventually rises to the surface. Some have speculated that this is a naturally occurring mud volcano, but L. William Abel, an American drilling expert advising Lapindo Brantas, says he believes the mud flow results from a drilling breach of a deep, pressurized reservoir.

Abel, whose ABEL Engineering/Well Control Co., based in Houston, Texas, has been involved in containing well accidents worldwide, says the volume of mud flowing out of the ground—about 50,000 cubic meters per day—is unprecedented. The mud has spread over 240 hectares, swamping whole villages, factories, shrimp farms, and rice paddies. The first attempt to block the flow was stymied in early August when the site was threatened by the rising tide of mud.

Efforts to block the flow had to wait while workers erected a higher retaining wall around a work site about 500 meters away from the original well. On 18 September, a drilling crew started on the first of two relief wells that they hope will intercept the original well within the shale formation 1500 to 1800 m down. They will then pump in high-density drilling mud to hydrostatically plug the leak.

Abel says this is a standard drilling industry technique; he is confident it will work. "There has never been one blowout in the history of drilling that was too tough to fix," he says.

The land around the geyser, meanwhile, is subsiding as the underlying clay erodes. Some 1400 military personnel are building containment ponds to hold the mud and water that continues to flow to the surface. Local and national officials are considering diverting the gunk into a local river and the sea. But that would be "a big disaster for fisheries and tourist areas," says Eko Teguh Paripurno, a geologist who heads a disaster research center at the University of National Development in Yogyakarta. Until the geyser is capped, Indonesian officials face a difficult choice between fouling additional land or the sea.

–DENNIS NORMILE

Liquid landscape. Steam and water from a deep carbonate formation are spreading mud over villages near Java's coast.

CREDIT: SIGIT PAMUNGKAS/REUTERS

PLANT SCIENCE

Parasitic Weed Uses Chemical Cues to Find Host Plant

Dodder may be the bloodhound of the plant world. A plant that parasitizes other plants, it sniffs out its victim, Justin Runyon and his colleagues at Pennsylvania State University in State College report on page 1964. "This is a pretty cool example of plants behaving in a way most people think only animals behave," says Richard Karban, a community ecologist at the University of California, Davis. It's also an effective strategy: Dodder ranks among the U.S. Department of Agriculture's top 10 noxious weeds.

The work bolsters the notion that plants have a chemical language, an idea that's been hotly debated for the past 2 decades. "The results go a long way toward convincing people that plant-plant interaction via volatiles is a real phenomenon," says Eran Pichersky, an evolutionary biologist at the University of Michigan, Ann Arbor.

Barely able to carry out photosynthesis, dodder survives by attaching to the stems and leaves of other plants and robbing them of nutrients. A relative of morning glories, it has many names—goldthread, strangleweed, witches' shoelaces—that aptly describe the dense, yellowish mats that blanket its hosts, reducing agricultural productivity by as much as 90%.

Runyon, a graduate student working with Pennsylvania State chemical ecologists Consuelo De Moraes and Mark Mescher, wanted to know how dodder found its mark. He placed seedlings of the dodder species *Cuscuta pentagona* in small vials fitted with a collar of filter paper on which he traced their growth and found that 80% of them headed toward a tomato plant placed nearby. He then put seedlings in an open-air chamber with two 90-degree side tunnels, one of which led to a chamber containing four tomato plants and the other to a chamber with four artificial plants; the seedlings could catch a whiff of the plants through the tunnel. About 77% of the seedlings grew toward the actual plants, the group reports. Runyon then replaced the plants with a vial of plant extract and the fakes with a vial containing only solvent. Again, the dodder seedlings made the right choice.

The seedlings also grew toward touch-me-not plants (*Impatiens*) and, to a lesser extent, wheat. But when given the choice, the dodder avoided wheat, a poor host, in favor of tomato. Runyon discovered that wheat emits a chemical that somewhat repels the dodder—a finding with possible practical implications, given that dodder is so hard to control. This result, says De Moraes, "suggests the possibility of using volatiles to enhance plant defenses, either by applying repellent compounds or perhaps by engineering plants to produce them."

Gotcha. A plant that preys on other plants, dodder winds its way up its host.

CREDIT: COURTESY OF THE DE MORAES AND MESCHER LABS

Past studies have indicated that plants under attack from herbivores emit signals telling nearby plants to boost their chemical defenses. But some researchers have been dubious about this evidence of plant-plant chatter, arguing that the experiments took place in closed chambers where artificially high concentrations of odors built up. Runyon's experiments were "rigorously conducted in an 'open' experimental design, so it's hard to argue that the responses they observe in the greenhouse are not occurring in the real world," says Ian Baldwin of the Max Planck Institute for Chemical Ecology in Jena, Germany.

Many questions remain about how plants perceive the still-unidentified volatile signals. But there will be rapid progress, predicts Andre Kessler, a chemical ecologist at Cornell University. Runyon and his colleagues, he says, have "opened up a new door that can bring us closer to the understanding of airborne plant-plant interactions."

–ELIZABETH PENNISI

CDC Employees Sound Off

An anonymous Web-based tip line for addressing low morale at the U.S. Centers for Disease Control and Prevention (CDC) has logged 75 comments since it was launched last week by congressional Representative Henry Waxman (D–CA). "We were surprised" at the number of responses, says Karen Lightfoot, a spokesperson in Waxman's office. More than a dozen high-level officials have left CDC since 2004, and the agency is reorganizing. Waxman started the tip line to get insiders' views of "the potential impact on public health" of the changes, Lightfoot says. CDC spokesperson Thomas Skinner says, "We certainly want to be supportive of efforts that further open the lines of communication." **–JENNIFER COUZIN**

Boston to Regulate Biosafety Labs

The Boston Public Health Commission has approved the first-ever municipal law regulating research on high-risk infectious agents. The rules, which come as Boston University (BU) plans the area's first biosafety level (BSL) 4 lab for the most dangerous pathogens, were scaled back last week after Harvard University and companies protested that they covered routine work in lower-risk labs. The final version applies only to BSL 3 and BSL 4 labs, which will have to get a permit, form a safety board including two community members, and file regular reports. "The regulation strikes a reasonable balance," says Kevin Casey, head of government relations for Harvard. The law bans classified work and research aimed at making bioweapons, topics not planned for the BU lab.

–JOCELYN KAISER

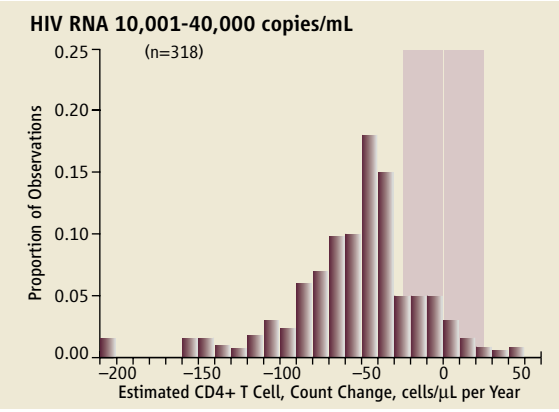
Familiar Tune for New KAIST Leader

SEOUL, SOUTH KOREA—Nam-Pyo Suh, the new president of the Korea Advanced Institute of Science and Technology (KAIST) in Daejeon, has announced reforms that parallel moves that got his predecessor, Robert Laughlin, a Nobel Prize–winning physicist, in hot water with faculty members (*Science*, 7 April, p. 32). Like Laughlin, Suh hopes to expand KAIST's mission beyond basic science by adding institutes of information technology, biotechnology, design, and entertainment engineering. And like Laughlin, he would charge students tuition—but only if they receive poor grades. Unlike his predecessor, Suh, formerly a professor of mechanical engineering at the Massachusetts Institute of Technology, would double KAIST's 400-member faculty, hiring as many as 100 non-Koreans. **–D. YVETTE WOHN**

IMMUNOLOGY

Study Says HIV Blood Levels Don't Predict Immune Decline

HIV levels in the blood are poor predictors of the rate at which the virus is destroying an infected individual's immune system, says a new study of nearly 3000 people. This challenges influential reports from a decade ago that link so-called plasma viral load levels to the onset of AIDS and death. The new work, published in the 27 September *Journal of the American Medical Association* (*JAMA*), also reinvigorates long-standing, contentious debates about how best to use viral loads in clinical care and about the mechanisms driving HIV's destruction of the immune system. "It's important because it means that things are not as obvious as we previously thought," says Daniel Douek, an immunologist at the U.S. National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland, who studies HIV pathogenesis. "There's more going on than just viral load causing problems."



Out of range. The rate of CD4 decline varies widely within a group of untreated HIV-infected people who have similar viral loads at first visit.

Benigno Rodríguez and Michael Lederman of Case Western Reserve University in Cleveland, Ohio, headed the study, which analyzed two separate cohorts of HIV-infected people to see how well an untreated person's first recorded levels of HIV RNA predicted immune destruction, as gauged by the annual loss of critical CD4 white blood cells. Their data simultaneously confirmed and challenged what has become dogma.

Confirming a landmark 1996 study published in *Science*, the researchers report that groups of people with higher viral loads lost more CD4 cells each year. But on an individual basis, viral load accurately predicted a person's CD4 decline just 4% to 6% of the time. "It really nicely illustrates that when you look at cohorts and find a general phenomenon—yeah, virus is high and CD4 is low—it can be very, very poorly accountable when you look at individuals," says immunologist Anthony Fauci, who heads NIAID. Lederman says this disconnect reflects the fact that if people are grouped by different viral loads, there is a "huge overlap" in CD4 loss between the groups.

Virologist John Mellors of the University of Pittsburgh in Pennsylvania, who led the team that published the 1996 *Science* paper, doesn't buy the conclusion. "We don't agree with the paper at all," says Mellors. "HIV RNA is the most powerful predictor of time to AIDS and death." He suggests that the *JAMA* paper's results may reflect that CD4 measurements vary a great deal in different labs. He also contends that viral load levels should continue to play an essential role in determining when to start people on treatment.

In another controversial twist, the *JAMA* paper and an accompanying editorial each note that the disconnect between individual viral loads and immune destruction supports the idea that only a small proportion of CD4 loss is due to direct killing by HIV. Rather, the authors contend that HIV causes immune mayhem mainly by over-"activating" the entire system. Once activated, cells self-destruct, leading many HIV-*un*infected CD4s to an early grave. Both Fauci and Douek agree with this assessment. "We must not forget that the virus is at the heart of this," says Douek. "But immune activation is probably more important than viral load in terms of rates of progression."

Virologist David Ho, head of the Aaron Diamond AIDS Research Center in New York City, questions that conclusion because he says the study leaves out a critical parameter: that people replenish CD4s at different rates. "Think kinetically," says Ho, noting that a person's bank balance is determined both by income and expenses.

The most "exciting implication" of the findings, says the editorial, may be an increased effort to test treatments that try to tamp down activation, augmenting the current strategy that just targets HIV.

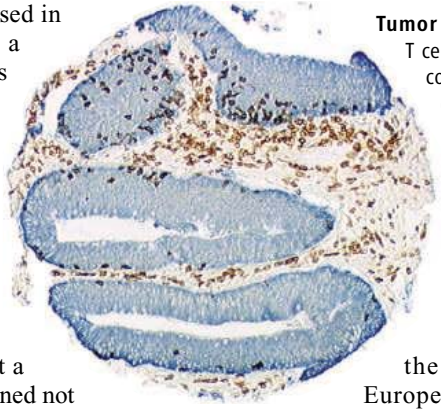
–JON COHEN

CANCER

T Cells a Boon for Colon Cancer Prognosis

Patients with early-stage colorectal cancer have an upbeat prognosis, and many don't even receive chemotherapy. But 30% will relapse, challenging the tenet that grapesized tumors that haven't spread aren't an undue cause for concern. Reaching back to a theory proposed in the late 19th century, a team of French scientists now says that in that 30%, recurrence may have little to do with the tumor itself. Rather, poor prognosis could stem from a lackluster immune reaction to cancer.

In 1889, a British surgeon proposed that a cancer's path is determined not just by qualities intrinsic to tumor cells—the "seed"—but also by the "soil," the environment in which a cancer grows. Attention has focused on the first, however, and treatment for virtually all solid cancers, like those in the breast, lung, and colon, is based on the tumor's size and whether it has spread. With the advent of genetics, dozens of papers have reported differences in gene expression in tumor cells and sought to tie prognosis to these patterns, with mixed success.



Tumor in check? A patient with T cells (brown) swarming near a colorectal tumor (blue) has a good shot at survival.

Immunologists Jérôme Galon of the Institute for Health and Medical Research in Paris, Franck Pagès of the Georges Pompidou European Hospital, and their colleagues took a different tack. Guided by their earlier work suggesting a link between metastasis and a weak immune response, the group hunted for T cells in the vicinity of colorectal tumors. They relied ▶

CREDITS (TOP TO BOTTOM): ADAPTED FROM B. RODRÍGUEZ *ET AL.*, *JAMA* **296**, 12 (2006); J. GALON *ET AL.*, *SCIENCE*

on banked samples from 415 patients collected over the past 16 years, along with information about how those patients had fared. They focused their search on the T cells that attack bacteria, viruses, and other pathogens and those that remember enemies they've encountered before.

Their findings, reported on page 1960, surprised even them: The density of T cells near tumor cells was, in these patients, a better predictor of survival than traditional staging based on a cancer's size and spread. "It's an incredibly great step forward," says Robert Schreiber, an immunologist at Washington University School of Medicine in St. Louis, Missouri. "One of the remaining arguments has been, 'Does this really occur in humans?' … The answer here is, 'Yes, it does.'"

Galon, Pagès, and the others divided their samples into two groups, depending on whether immune cell concentrations were high or low. Patients whose tumors brimmed with CD3-positive T cells, for example, had a 5-year survival rate of 73%, compared with 30% for patients with low densities of CD3 cells around the tumor. In those with earlier-stage tumors, the spread was still clear: High densities of CD3 T cells correlated with a 79% chance of survival after 5 years. With low densities, the proportion was 33%. It's not known yet why some patients have more robust immune responses and whether tumor types help drive the difference.

"The more T cells are infiltrating these lesions, the better survival is," says Karin de Visser, a tumor immunologist at the Netherlands Cancer Institute in Amsterdam. It's often believed that a heightened immune system drives cancer rather than squelching it. For instance, conditions that induce chronic inflammation, such as colitis, seem to confer a greater cancer risk. But this work suggests that once a cancer forms, the immune system can also help beat it back.

De Visser and others agree that the research needs to be repeated in larger groups of colorectal cancer patients and in those with other cancers. There have been hints in melanoma that T cells can hinder that cancer's progression; but in breast cancer, says de Visser, a handful of cases suggest that the opposite may be true.

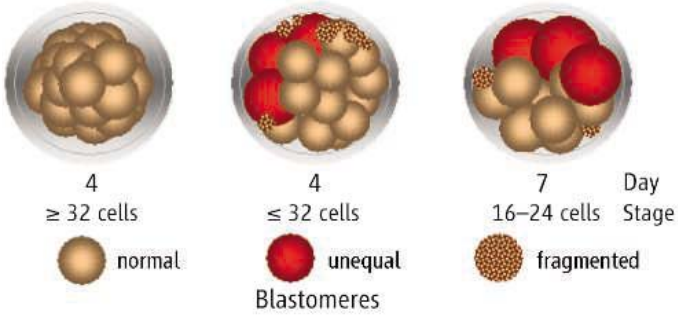
More immediately, Galon hopes that if the results hold, T cell densities could help identify apparently low-risk individuals who would benefit from aggressive treatment. "Today there is no way to predict those patients who will have a bad outcome" in this cohort, he says. In theory, the finding could also lead to new treatments that stimulate these T cells to proliferate and contain a tumor. **–JENNIFER COUZIN**

CREDIT: X. ZHANG *ET AL.*, *STEM CELLS EXPRESS* (2006, ALPHAMED PRESS)

STEM CELLS

Scientists Create Human Stem Cell Line From 'Dead' Embryos

Scientists are working feverishly on methods to create lines of human embryonic stem (ES) cells that do not involve the destruction of human embryos. Last week, a European team reported that it had done just that, cultivating a line of stem cells from an "arrested" or nonviable embryo. They and others say that the technique could satisfy people who object to stem cell research on the ground that it harms potential human life—although others are skeptical that there can be a universally accepted definition of "arrested."



Under arrest. Normal embryo (*left*) has more and more-uniform cells than do those that have stopped growing.

Donald Landry and Howard Zucker of Columbia University proposed several years ago that embryos that had stopped dividing might still yield individual cells that could be induced to grow separately. Now a team headed by biologist Miodrag Stojkovic, who has labs in Valencia, Spain, and at Sintocell in Leskovac, Serbia, reports that it generated a pluripotent ES cell line—that is, cells that can develop into all types of bodily tissues—from one of 13 embryos that had stopped developing 6 to 7 days after fertilization, at the blastocyst stage. The researchers waited from 24 to 48 hours after the last cell division to determine that the embryos, donated for research, were no longer viable, they reported online in *Stem Cells* on 21 September.

They then removed the zona pellucida, or outer covering, of embryos and plated them on a growth medium. In five of the 13 cultures, some cells proliferated. In one case, the scientists were able to achieve a "fully characterized" human ES cell line that could differentiate into all three germ layers both in the dish and in live mice. Attempts to cultivate ES cell lines from another 119 embryos that were arrested at an earlier stage—as 4- to10-cell morulas—were not successful.

Stojkovic's team claims that as many as two-thirds of in vitro fertilization embryos fail to reach the stage at which they can be implanted. Now, they say, scientists will be able to use this material that would otherwise be discarded. The authors assert that their approach is more efficient than one reported last month by scientists at Advanced Cell Technology (ACT) in California: deriving an ES cell line from a single cell taken from a morula (*Science*, 25 August, p. 1031). The ACT authors were criticized for claiming their procedure could be done without harming embryos when in fact they destroyed those used in their experiments.

The new study "offers hopeful possibilities" for getting the National Institutes of Health, which does not permit harm to live embryos, to fund research on newly derived ES cell lines, says Stanford University bioethicist William Hurlbut. "But scientists have yet to arrive at reasonable criteria for 'embryo death'"; 24 hours without cell division may not be enough." Landry agrees that "explicit definitions for irreversible arrest" of embryo growth are still lacking and is working to come up with watertight criteria.

With the recent papers from ACT and Stojkovic's group, scientists are bringing closer to reality various alternatives to embryo destruction that a few years ago were only ideas. Last year, a group at the Massachusetts Institute of Technology showed that a technique called altered nuclear transfer, involving the creation of an embryo incapable of developing, is feasible in mice. And a group at the University of Milan has claimed in as-yet-unpublished work that it has generated human ES cell lines by parthenogenesis, using only unfertilized eggs.

–CONSTANCE HOLDEN

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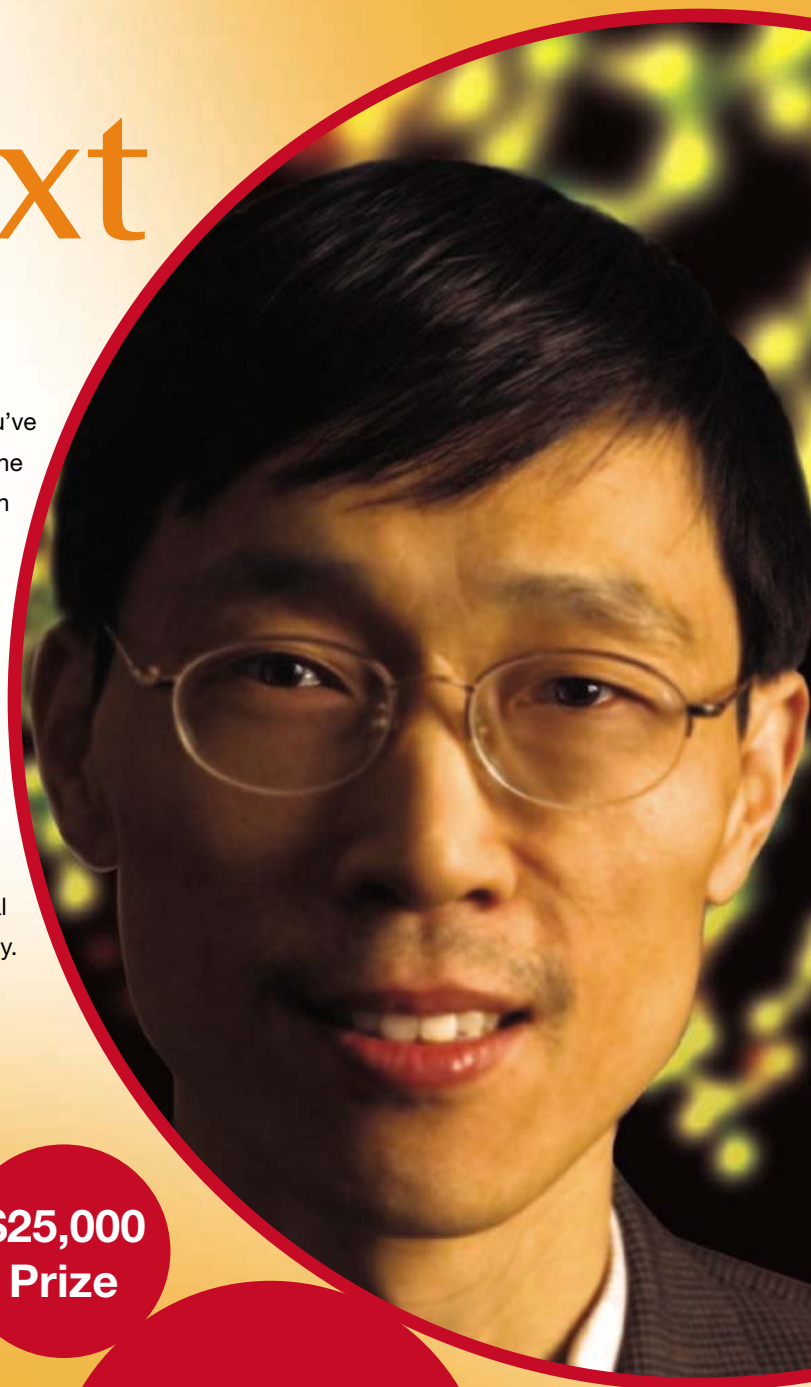

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CLIMATE CHANGE

Royal Society Takes a Shot at ExxonMobil

The world's oldest scientific society has challenged the world's richest corporation over what it sees as an attempt to confuse people about global warming. In a sharply worded letter made public last week, the 346-year-old Royal Society criticizes the oil giant ExxonMobil for giving money to "organizations that have been misinforming the public about the science of climate change" and for promoting an "inaccurate and misleading" view, to wit: that scientists do not agree about the influence of human activity on rising temperatures. ExxonMobil issued a rebuttal, and some climate-change skeptics attacked the Royal Society for trying to stifle debate.

The letter, drafted by Royal Society Senior Manager for Policy Communication Bob Ward and approved by the society's leadership, was sent to ExxonMobil's U.K. director of corporate affairs. It charges that the company's *Corporate Citizenship Report* and a February 2006 strategy booklet called *Tomorrow's Energy* are "very misleading." In one passage, the company dismisses the work of the Intergovernmental Panel on Climate Change (IPCC) as based on "expert judgment rather than objective, reproducible statistical methods." The ExxonMobil documents claim it is "very difficult to determine objectively the extent to which recent climate changes might be the result of human actions." These statements, the Royal Society says, are "not consistent with the scientific literature."

Ward also questions ExxonMobil's funding of groups that deny the connection between carbon dioxide and climate change. For example, he told *Science* that the corporation last year gave $25,000 to the Center for the Study of Carbon Dioxide and Global Change in Tempe, Arizona. On its Web site, the center claims "there is no compelling reason to believe that the rise in temperature [since the industrial revolution] was caused by the rise in CO_2." According to the Royal Society, "some 39 organizations" listed as beneficiaries on ExxonMobil's own Web site give out misleading information. Ward says that when he met with company officials in July, they said they planned to discontinue support for such groups. This month's letter, he says, was an effort to "follow up."

ExxonMobil spokesperson David Eglinton responded that the Royal Society described the company "inaccurately and unfairly." A company statement says, "We know that carbon emissions are one of the factors that contribute to climate change," and "ExxonMobil is taking steps to reduce and minimize … greenhouse gas emissions." It notes that the company has sponsored a major climate research project at Stanford University. The funding of other policy groups is in review, ExxonMobil says.

> "While assessments such as those of the IPCC have expressed growing confidence that recent warming can be attributed to increases in greenhouse gases, these conclusions rely on expert judgment rather than objective, reproducible statistical methods."
>
> —ExxonMobil, *Tomorrow's Energy*

Several groups or individuals who contest the IPCC view of climate turned fire on the Royal Society. The George C. Marshall Institute in Washington, D.C., distributed critical remarks by several experts, including atmospheric scientist William Gray, a professor emeritus at Colorado State University in Fort Collins. "I am appalled … that the Royal Society would try to muzzle" skeptics, Gray says. Economist Ruth Lea, director of the conservative Centre for Policy Studies in London, says that the Royal Society was "ill advised" to "wade into the murky world of politics and popular opinion."

Ward replies that his letter is merely an attempt to ensure a high-quality debate. He adds that it springs from the Royal Society's motto—*nullius in verba*—which is taken to mean that facts, not assertions, are what matter.

–ELIOT MARSHALL

ASTRONOMY

Search for Giant Scope Site Narrows to Two

The Square Kilometer Array (SKA)—planned to be the world's most powerful radio telescope—will be built in either Australia or in southern Africa. The two other site contenders, Argentina and China, were eliminated on technical grounds, according to an announcement made this week by the SKA steering committee. The final site decision is not expected for several years, with construction starting 2 years after the final choice if international funders agree to back the estimated $1 billion project (*Science*, 18 August, p. 910).

SKA's international director, astronomer Richard Schilizzi, said both short-listed sites "can meet the full range of requirements" for the instrument, which will link thousands of antennae spread over 3000 kilometers. Perhaps the most important requirement is low radio interference. U.K. astronomer Phil Diamond, a former steering committee chair, said both potential sites are making progress toward "protecting these unique environments with radio-quiet zones" through laws or regulations.

The Argentina site was rejected mainly because of ionospheric instability; China's proposed site amid karst hills did not offer the right geography to position SKA's central elements. Bo Peng of China's National Astronomical Observatories in Beijing described the decision as being "fair from the scientific point of view."

South Africa's SKA chief Bernie Fanaroff says his group would like the final site selection to be made as soon as feasible, but his Australian counterpart Brian Boyle says that SKA should avoid setting "an unrealistic time scale" that doesn't allow time to finalize the design, build infrastructure, and line up international funding. Schilizzi says he expects the final site decision "towards the end of the decade," allowing time to raise the money and prepare the site for construction. Some doubts remain about funding, with the U.S. National Science Foundation so far avoiding a firm commitment. But Boyle says he is confident that SKA will eventually be built: "It is too good of an astronomy opportunity to pass up."

–ROBERT KOENIG

The U.S. Department of Energy redirects its big machines toward small-scale research, as materials science overtakes particle physics

Embracing Small Science in a Big Way

Brilliant! The world's first x-ray laser, shown in this artist's depiction, will open new avenues of research in materials science—and close a chapter in the history of high-energy physics at SLAC.

CREDIT: COURTESY OF STANFORD LINEAR ACCELERATOR CENTER

THE HEFTIEST BUILDING BLOCKS OF nature were discovered at the Stanford Linear Accelerator Center (SLAC). In 1967, researchers at the Menlo Park, California, laboratory shot electrons from a 3-kilometer-long "linac" into protons and detected the first hints of smaller particles within. Those infinitesimal bits of matter, known as quarks, are now a cornerstone of the so-called Standard Model, which describes how one type of quark decays into another. Nearly 40 years later, high-energy physicists still use the linac to feed particles into a collider, called PEP-II, with which they compare quarks and antiquarks.

But in 2008, PEP-II will shut down, and a year later, researchers at SLAC will power up the world's first x-ray laser, the Linac Coherent Light Source (LCLS). It won't produce exotic subatomic particles that might allow researchers to peer further into the fundamental structure of matter. Instead, by shining a billion times brighter than other x-ray sources, LCLS will probe the properties of familiar materials in unprecedented ways, determining, for example, the structure of a protein from a sample of just one molecule. "The LCLS will be revolutionary," says Persis Drell, SLAC's deputy director for particle physics.

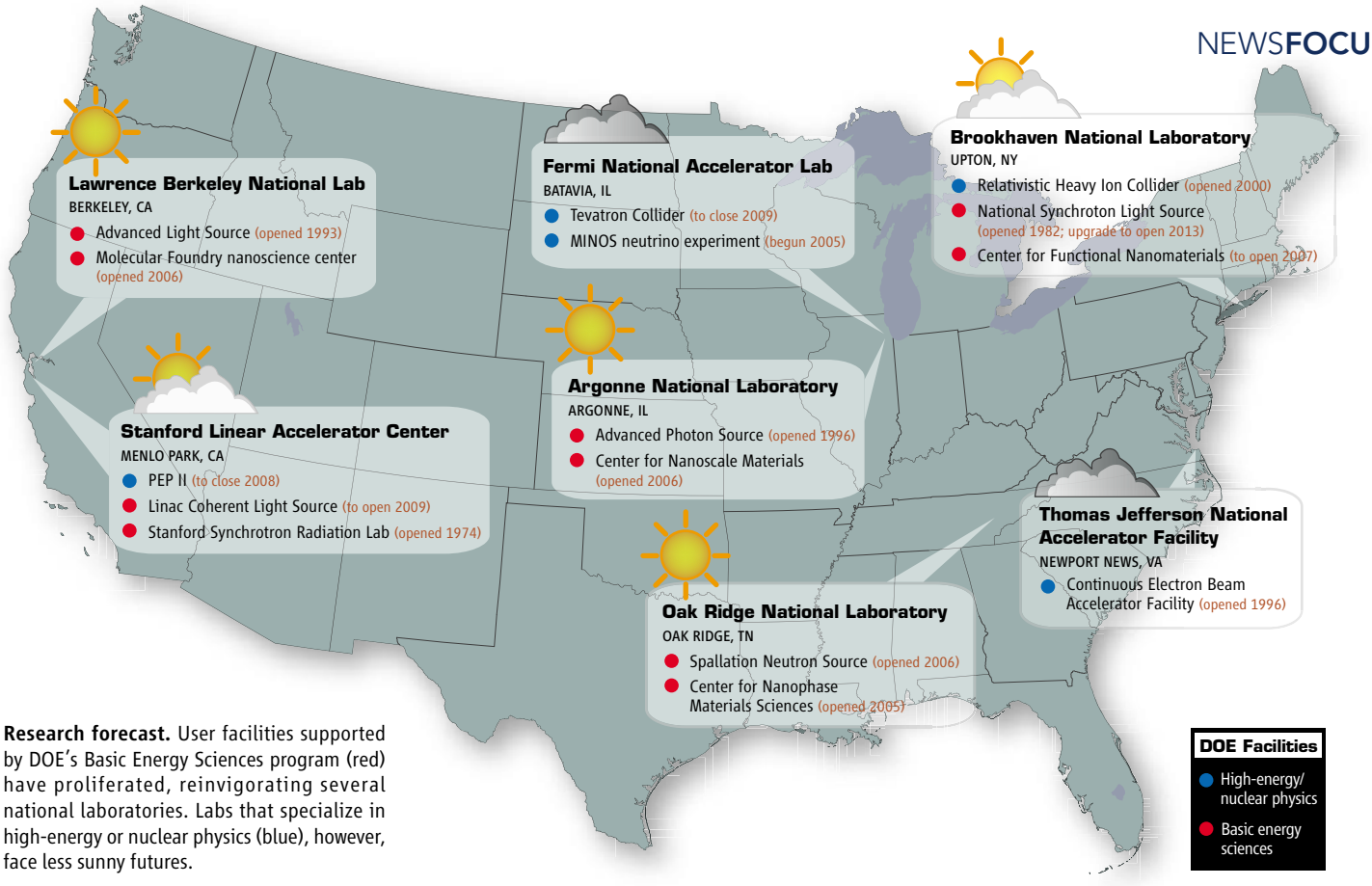
To nonphysicists, the lab's shift from elementary particles to materials may not seem like a big deal. But it's a prime example of the change sweeping over the Department of Energy's (DOE's) $4 billion Office of Science, which runs 10 national labs, funds thousands of academic researchers, and provides 42% of the U.S. government's overall funding for the physical sciences.

For half a century after World War II, nuclear and high-energy particle physics ruled the roost. DOE-funded scientists have captured 23 Nobel prizes for exploring the composition of the universe and other grand academic puzzles. In the past 2 decades, however, the Office of Science has directed ever more resources to the down-to-earth fields of materials sciences, condensed-matter physics, chemistry, and nanotechnology, all supported by its Basic Energy Sciences (BES) program. Leveraging its expertise in accelerator physics, DOE has built a suite of large "user facilities" that allow researchers to study a vast array of materials, catalog thousands of protein structures, and even analyze the respiration of insects. Its $1.4 billion budget makes BES by far the office's largest program.

The shift in priorities, which reflects the emerging opportunities in the physical sciences themselves, is changing the role and the culture of the DOE science labs. Once the cloistered preserves of a small coterie of scientists pursuing the most esoteric questions, the DOE labs now serve as gathering places where thousands of researchers from many fields perform myriad studies. "You have experiments coming and going every week and every few hours," says Lonny Berman, an x-ray physicist at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory in Upton, New York. "It's like Grand Central Station."

Small science, big machines

The impact of the shift in DOE science can be seen clearly at Oak Ridge National Laboratory in the fir-covered foothills of the Smoky Mountains in eastern Tennessee. Once considered a backwater, the lab is becoming a mecca for materials scientists thanks to two new user facilities. In April, the lab fired up its $1.4 billion Spallation Neutron Source (SNS), a sprawling accelerator complex that produces intense pulses of high-energy neutrons for probing materials. Nearby stands the newly opened Center for Nanophase Materials Sciences, one of five nanoscience centers DOE is building around the country. "It's a tremendously enabling facility," says Philip Rack, a materials scientist at the University of Tennessee, Knoxville, who collaborates with Oak Ridge researchers.



Research forecast. User facilities supported by DOE's Basic Energy Sciences program (red) have proliferated, reinvigorating several national laboratories. Labs that specialize in high-energy or nuclear physics (blue), however, face less sunny futures.

SNS is the latest in a string of accelerator-based facilities aimed at small science. In the 1980s, DOE began building synchrotrons to generate x-rays—which the circulating particles radiate in copious amounts—to probe the structures of materials. The first was at Brookhaven. It was followed by one at Lawrence Berkeley National Laboratory (LBNL) in California and another at Argonne National Laboratory in Illinois. Labs that had once focused on particle physics had new missions. The various BES user facilities are "the envy of the world," says Raymond Orbach, head of the Office of Science and the agency's first undersecretary for science (see sidebar on p. 1874).

The BES program has grown even as DOE's Office of Science has trimmed or held steady its programs in high-energy physics, nuclear physics, fusion-energy science, and biological and environmental research. In addition to the compelling scientific opportunities, observers credit the leadership of Patricia Dehmer, who has headed BES since 1995 (see sidebar). "Within the Office of Science, the competition is between the associate directors," says a congressional staffer who follows DOE science. "And Pat Dehmer is just a smarter player."

The new machines have also changed the culture of science at the host labs. At particle physics labs such as SLAC or Fermi National Accelerator Laboratory (Fermilab) in Batavia, Illinois, hundreds of physicists work for years on a single experiment. And experimenters and accelerator physicists typically work hand in hand to improve the performance of the machine they share.

At an x-ray source, most users come for just a few days at a time and have no interaction

A Manager Who Cashes In on Consensus

Patricia Dehmer was hoping to get 75 basic researchers to attend a workshop on solar power. Instead, 200 scientists signed up, and the head of the Department of Energy's (DOE's) Basic Energy Sciences (BES) office needed to book a second hotel to handle the overflow. It's what she calls finding "a latent community" of scientists ready to tackle a new problem. And it's one of the techniques that she has used successfully over the past 11 years to more than double BES's budget, turning it into a $1.4 billion juggernaut (see main text).

"These [initiatives] would have been tough sells had the community not come together," says chemical physicist John Hemminger, chair of the BES Advisory Committee, about the 2005 solar-power workshop and similar gatherings on hydrogen and lighting. The community's strong support for those nascent initiatives, in turn, helped grease the budget skids with higher-ups at DOE, in the White House, and in Congress. "You have things ready and well-justified, and when the time is right, they go," says Dehmer, a chemical physicist who came to DOE headquarters in Washington, D.C., in 1995 from DOE's Argonne National Laboratory in Illinois.

Dehmer has also learned how to stagger projects so that they don't overwhelm her budget. In doing so, she shepherded the completion of the $1.4 billion Spallation Neutron Source at Oak Ridge National Laboratory in Tennessee and four of five nanotechnology centers at other DOE national labs on time and on budget.

A former DOE colleague, Ari Patrinos, ruefully admits how Dehmer's "highly organized" approach steered resources to BES—at times at the expense of his Office of Biological and Environmental Research. "The way she times the projects is masterful," says Patrinos, now at Synthetic Genomics in Rockville, Maryland. A tireless commitment to her job hasn't hurt either, Patrinos adds: "I remember e-mails from her at 3:40 a.m.; she was perpetually working." **–E.K.**

CREDITS (TOP TO BOTTOM): DEPARTMENT OF ENERGY, OFFICE OF SCIENCE; DOE

Ray Orbach Asks Science to Serve Society

For a decade, chemist Radoslav Adzic has explored the basic structure of metal-electrolyte interfaces at Brookhaven National Laboratory in Upton, New York. His employer, the U.S. Department of Energy (DOE), has long sponsored fundamental science on catalysis in such systems in hopes of making hydrogen fuel cells efficient enough to one day replace fossil fuels as an energy source. But it wasn't until 2004 that Adzic decided to tackle a research question with more direct applications: how to use monolayers of platinum to build cheaper fuel cells, focusing on hydrogen.

It wasn't a random decision. The year before, President George W. Bush had proposed an 8-year, $1.2 billion hydrogen fuels program that would begin with applied engineering studies. After attending a DOE-sponsored workshop to discuss the basic research needed to turn hydrogen into a commercially viable fuel, Adzic won a $700,000 grant to study how monolayers of platinum could lead to cheaper fuel cells. Although it's still basic research, there's a clear product in mind.

That program, and others like it, reflects changes sweeping through DOE's $4-billion-a-year Office of Science under the leadership of Raymond Orbach. A physicist and former chancellor of the University of California, Riverside, Orbach joined DOE in 2002 and has won praise for grafting an applied component onto DOE's basic science portfolio without diluting the quality of the research itself. Michael Lubell, a lobbyist for the American Physical Society, calls the hydrogen funds "a testament to Ray" and praises his success in enlarging the pot for such work without ceding control of it to more technology-focused parts of DOE. Adzic agrees: "Rather than just characterizing a system, I'm helping to solve certain showstoppers."

Bolstering research on hydrogen fuels is only one of Orbach's achievements. He's also brought international renown to DOE's once-puny civilian supercomputing program and made progress on lab safety procedures. And physical scientists applaud how he helped his boss, Energy Secretary Samuel Bodman, sell the White House on a 10-year doubling of federal spending in the physical sciences by emphasizing its role in U.S. competitiveness (*Science*, 17 February, p. 929).

In June, Orbach donned a second hat by becoming the department's first undersecretary for science. His mission: Optimize the research goals that span DOE's work in energy, science, weapons, and waste by offering the fruits of the Office of Science's basic research portfolio to the rest of the agency. "There isn't a single thing that DOE does that's not grounded in science. [So] having someone who can ask scientific questions [about] each of the missions is crucial," says David Goldston, staff director of the House Science Committee. "He's the perfect person for the job."

Keys to the kingdom. No one doubts that fundamental research could better fulfill energy needs. A 1997 report by the President's Council of Advisors on Science and Technology, for example, called for "better coordination" between basic and applied energy research. "Everyone knows it's a problem, but nothing's happened," says physicist George Crabtree, a manager at DOE's Argonne National Laboratory in Illinois.

One obstacle is the current rewards system in academia. Take the science behind superconductivity, which holds the promise of low-resistance power lines or incredibly efficient transformers. The kind of discovery that earns a scientist a paper in a top journal—learning why a material changes phase at a certain temperature—is too theoretical to help a company trying to make superconducting materials. But a commercially valuable yet incremental improvement in that technology wouldn't interest those top-tier journals. So a scientist might not even bother to record such an advance. "If the currency is just *PRL* [*Physics Review Letters*], *Nature*, and *Science*, you'll just move on," says materials scientist John Sarrao of Los Alamos National Laboratory in New Mexico.

Another barrier to developing new technologies, says Bodman, is DOE's current compartmentalized bureaucracy. In July, he sent out a memo giving Orbach "detailed access" to DOE's vast empire, hoping that regular meetings among disparate programs will break through that mentality. It's not a new concept, Orbach says, but "what's new is the intensity and importance" of those meetings.

Money greases the wheels of cooperation. In addition to the hydrogen initiative and a similar effort in solar energy, Orbach has called for $250 million for biofuel start-ups involving industrial scientists, technologists, and genomicists (*Science*, 11 August, p. 746). Sharlene Weatherwax, a DOE program manager, says a previous partnership with DOE's technology program might have consisted of a single grant.

Orbach knows that change doesn't come easily for areas, such as nuclear weapons development, that have traditionally been walled off from civilian research. In initial meetings with applied-research managers, he admits, "people don't quite know what to make of us." But Edward Moses, director of the National Ignition Facility, a superlaser at Lawrence Livermore National Laboratory in California, says Orbach is helping him grow a civilian research community to utilize an instrument designed to maintain the nation's nuclear arsenal.

Some fear that such cross-fertilizing could weaken basic science at DOE. "There is a danger of letting the basic program become a technical-support enterprise for the applied programs," says energy expert Robert Fri, a former Environmental Protection Agency official who believes unfettered basic work can "cook up" whole new energy ideas. Materials scientist Ward Plummer of the University of Tennessee, Knoxville, decries a 20% decline in funding core, unsolicited research within DOE's Office of Basic Energy Sciences in the last 3 years at the same time that solar energy, nanotechnology, and hydrogen programs have grown.

Teammates. Ray Orbach (*left*) hopes researchers can help his boss, Energy Secretary Samuel Bodman, (*right*) do his job, too.

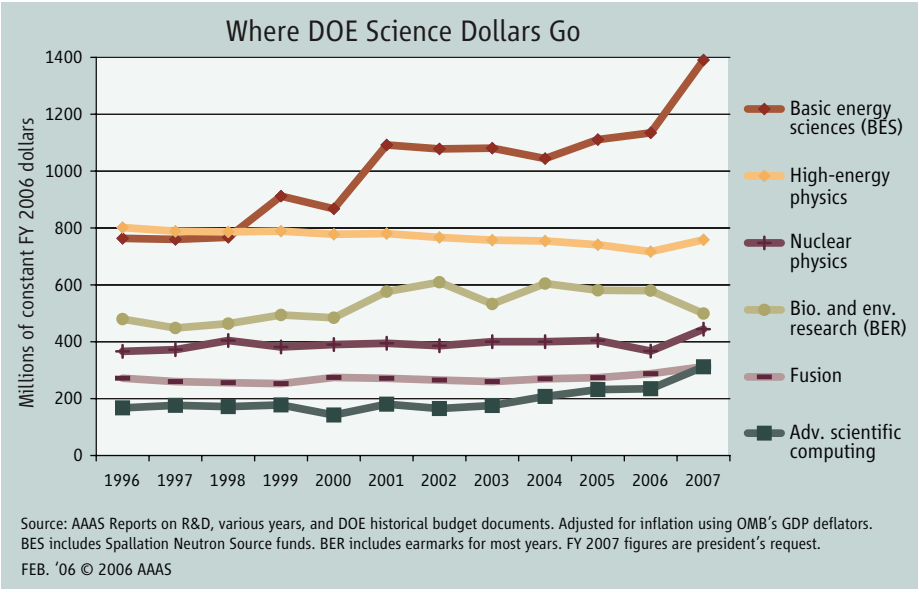
CREDIT: DOE

Plummer and others hope that DOE's new effort to define so-called grand challenges will stop that erosion. And although Orbach says he has no plans to "fuzz the boundaries" between basic and applied work, he is looking for greater cooperation between the two camps. A recent discussion with managers studying how fluids flow in dry soil at DOE's planned nuclear waste fuel repository at Yucca Mountain, Nevada, proves its value, he says. "When we met with Fossil Energy and learned more about carbon dioxide sequestration," Orbach recalls, "it suddenly popped out that that's the same problem."

Whatever happens, Orbach says DOE is determined to squeeze more impact out of its science. That's good news for Adzic, who relishes taking on challenges "directly important to society." It's also a good deal for academics. "If you publish something relevant" to a problem, says Adzic, "your paper is more [often] cited." **–ELI KINTISCH**

with the accelerator physicists. That's especially true of biologists, who use the x-rays to decipher the structures of proteins. "We're the ultimate users," says Steven Almo, a protein crystallographer at the Albert Einstein College of Medicine in New York City who uses Brookhaven's NSLS. "We just want to go out there, get the data, come home, and solve the structure." That attitude irks some lab scientists, who say that it's much harder than in the old days to find users who are also interested in improving the machine.

The user facilities have sewn new ties between the labs and industry. At NSLS, ExxonMobil and IBM own and have outfitted seven of the synchrotron's 65 beamlines, and others pay to perform proprietary research there. "If the facility does its job right, it becomes a focal point for collaboration between industry, the national labs, and the universities," says Chi-Chang Kao, a solid state physicist at Brookhaven. For example, at Argonne's Advanced Photon Source, researchers from Harvard Medical School and Hawaii Biotech Inc. used a beamline owned by a consortium of pharmaceutical companies to determine how the dengue fever virus changes shape as it infects a cell.



Source: AAAS Reports on R&D, various years, and DOE historical budget documents. Adjusted for inflation using OMB's GDP deflators. BES includes Spallation Neutron Source funds. BER includes earmarks for most years. FY 2007 figures are president's request.

Putting basics first. Spending on the basic energy sciences within DOE's Office of Science has doubled—and soon could grow even faster—while spending for most other fields has remained flat.

A new model laboratory

A push to focus more on "use-inspired research" could lead to even more dramatic changes. With ATT's Bell Laboratories of old as his model, LBNL Director Steven Chu envisions multidisciplinary teams that can translate basic research into technology attacking large energy problems. One proposed project, called Helios, would pursue the biology, materials science, and nanotechnology necessary to efficiently convert solar power to liquid fuel, whether through the production of ethanol from cellulose, improved solar cells, or direct conversion of sunlight to fuel. Chu would like DOE to provide flexible "block grants" for such projects so that lab managers could quickly follow up on promising leads.

Such boundary-bridging efforts would be a break from current operating procedures for the national labs, in which researchers from various divisions in the same lab answer to different program managers at DOE headquarters in Germantown, Maryland. "They want to own you and to take credit for your work; that's how the system works," says Heinz Frei, a physical chemist at LBNL and a member of the Helios team. For crosscutting efforts to flourish, Frei says, "there has to be an understanding at headquarters" that such projects span program boundaries.

Michael Lubell, a lobbyist with the American Physical Society, says Orbach has been key in fostering such cooperation, especially now that his position as undersecretary for science gives him responsibility for applied research done in other parts of DOE. Orbach also works well with Under Secretary of Energy David Garman, who runs DOE's fossil energy, nuclear energy, and other applied nonweapons programs, Lubell says. But the bridge-building may falter once either leaves office, he says.

Scattered clouds

DOE hasn't turned its back on its high-energy and nuclear physics programs, requesting $775 million and $454 million for them, respectively, in its 2007 budget. But particle physicists at SLAC and Fermilab and nuclear physicists at Brookhaven and the Thomas Jefferson National Accelerator Facility in Newport News, Virginia, face a tougher struggle to win political support for billion-dollar projects that seek to understand the origin of mass or the nature of matter in the early universe. "I would think it would be a mistake on the part of high-energy and nuclear physicists to expect that, at least in the near future, they'll receive a fixed piece of the pie," says Robert Richardson, vice provost for research at Cornell University, who has served on various national advisory committees.

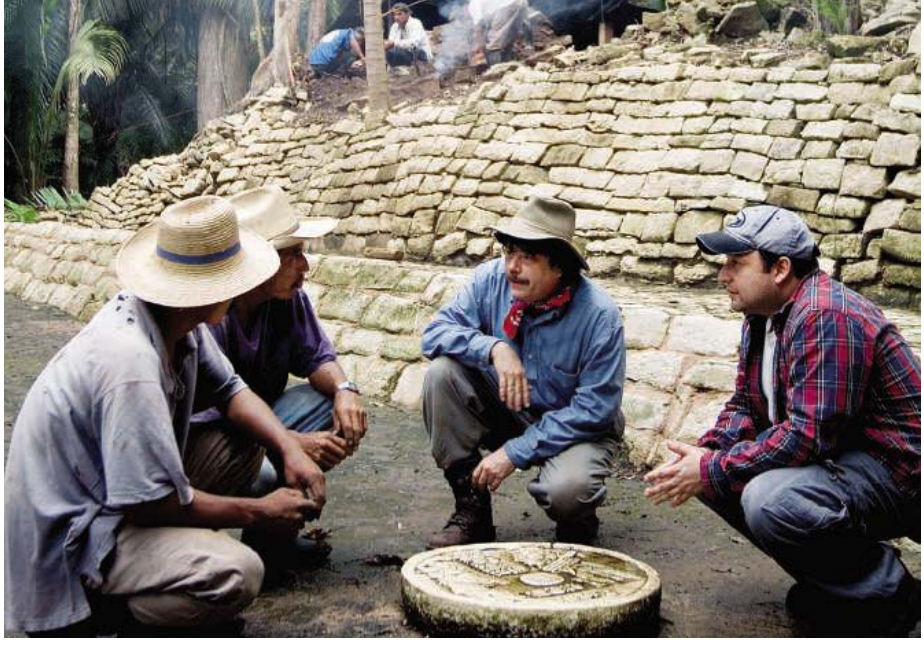
Despite its steady growth, DOE's BES program has also generated some controversy. Some researchers say that it funds too many groups and centers. "Lab stewardship has been equated with maintenance of a status quo that includes too many less-than-cutting-edge research teams," says one researcher who asked not to be identified because he receives BES funding. Many researchers say that DOE starves grant-seekers in tough times to keep the machines running. In recent years, the balance in the BES budget between grants and facilities has slipped from an even funding split to a 45:55 ratio, Orbach says. With the projected budget increases, DOE hopes to restore the balance.

Other researchers worry that BES has skimped on the basic accelerator research necessary to develop the next generation of user facilities. "BES has appropriately pushed its resources toward the users to get the [maximum] science out of the machines, and that doesn't require a lot of R&D," says Rod Gerig, an accelerator physicist at Argonne. Some researchers say that the United States already lags behind Europe and Asia, which are building a plethora of new x-ray sources.

Growing pains aside, basic energy sciences are on the rise. That's fine with many SLAC researchers, who view the lab's shift of mission as an opportunity to try something new. Accelerator physicist Franz-Josef Decker says that SLAC's x-ray laser will be so experimental that accelerator physicists and users will need to pull together just to make it work. "I don't think we'll turn it on and say, 'Okay, that's it,' " he says. Change is welcome, it seems, as long as it comes with a challenge.

–ADRIAN CHO

With reporting by Robert F. Service.



Courtside. Arthur Demarest (*center*), Tomás Barrientos (*right*), and local Maya gather in a restored royal ball court.

CREDIT: J. RABINOWITZ PHOTOGRAPHY

PROFILE: ARTHUR DEMAREST

Living Among the Maya, Past and Present

Archaeologist Arthur Demarest advocates passionately for studying the ancient Maya and for helping their living descendents in Guatemala

SAN JUAN, PUERTO RICO—Arthur Demarest strides into the seminar room, a good 10 minutes late for his talk on postcolonial approaches to archaeology and tourism. The moderator is about to introduce the next speaker but quickly yields the lectern to Demarest. Smartly turned out in a black Brazilian suit and a longish haircut, the archaeologist ignores his prepared remarks and eschews the lectern. Instead, he theatrically wanders back and forth in front of his audience at the Society for American Archaeology (SAA) conference here, gesturing and spicing his speech with expletives and evoking occasional laughter.

Demarest speaks passionately about his philosophy of using archaeological projects to better the lives of impoverished local people, a practice often referred to as community archaeology. "This is my baby," he says of his community work, but adds that Vanderbilt University in Nashville, Tennessee, where he is a professor, gives him grief because of it. Demarest criticizes many of his colleagues for not adopting this practice, then declares that discovering royal palaces (he's found several) bores him. "Sorry for the breathless presentation," he concludes.

Demarest is arguably one of the most successful proponents of community archaeology, with a string of dramatic discoveries on the Maya to his credit and a fundraising record—tied to his community efforts—that many might envy. "He's a visionary when it comes to [community] archaeology," says Raymond Chavez, vice president of Counterpart International, a nonprofit development organization based in Washington, D.C., that works with Demarest. "Everyone pretty much acknowledges Arthur's pioneering work" in Guatemala.

Although Demarest disparages the "Indiana Jones" style of archaeology, in some respects he resembles the onscreen archaeologist, battling looters and sometimes appearing with bodyguards. Some colleagues say he puts too much emphasis on public relations but agree that he's done much to popularize Mayan archaeology. "Arthur's work is significant because it raises the level of interest in all things Maya," says Maya scholar Arlen Chase of the University of Central Florida in Orlando.

Into the field

Demarest, 53, says he declared his intent to become an archaeologist at age 4, while growing up in New Orleans, Louisiana. He's the son of a Cajun inventor and the "world's greatest cook," he says. He earned a Ph.D. in the field from Harvard in 1981, studying under noted Mayanist Gordon Willey. He was offered a job at Harvard in 1986 but instead chose Vanderbilt. Vanderbilt gave him the resources to direct a huge project in the Petexbatún region in northwest Guatemala, and Demarest has been working in the country ever since.

For nearly 20 years, he has excavated sites along the great Maya trade route that ran north to south. He has given the field "important insights into ancient Maya warfare and trade," says Jeremy Sabloff of the University of Pennsylvania Museum of Archaeology and Anthropology in Philadelphia.

In Petexbatún, Demarest found evidence of warfare that engendered the collapse of the local Maya society. The findings, such as a shattered throne, are "among the most detailed and graphic for violence accompanying political collapse in Maya civilization," says archaeologist David Friedel of Southern Methodist University in Dallas, Texas. Demarest showed that the Maya collapse in Petexbatún was "likely precipitated by warfare driven by political and ideological factors rather than by population pressure and contingent agricultural failure," as some archaeologists had thought, says Friedel. Debate on the causes of the collapse continues for the rest of the Maya world, and since 1999 Demarest has worked at Cancuén, a medium-sized city on the trade route, where he has found a massive royal palace and evidence of a massacre.

For Demarest, the most important part of his work is the community component. He argues that too many archaeologists are seduced by what he calls the "goodies": palaces, tombs, and other spectacular finds. Temporary projects employ local workers, many of whom are descendents of the Maya, but when the digs end, the locals are suddenly unemployed and so may loot the site. Archaeology "is like finding an oil well. It can make everybody rich, or it can destroy the area," he says.

Demarest designed the Cancuén project to make the local people "stakeholders," and it seems to have worked. For example, the excavations have made Cancuén a minor tourist attraction, and the only way to reach the site is by water. So the Maya own and operate a boat service to ferry tourists, as well as a visitor's center and lodge. Overall, tourism at Cancuén has boosted their income by 35%, according to Chavez. The locals have reciprocated by telling Demarest where to dig and informing him about looting incidents. Using locals' knowledge has become part of his scientific methodology. "The distinction between development and science is gone," he says.

He says the Guatemalans consider him "a political figure," and in 2004, he became

the first American to be awarded the National Order of Cultural Patrimony by the Guatemalan government. The government has also adopted his community-focused philosophy, mandating that all archaeological projects in Guatemala include a community-development component. Chavez says Demarest was the first to implement a strategy that allowed the local Maya to profit from tourism to archaeological sites.

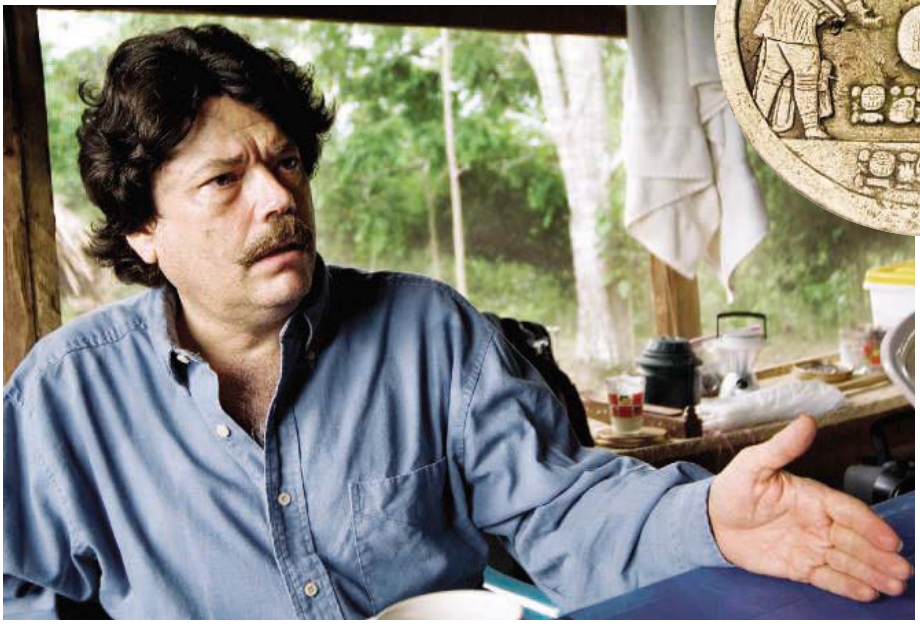
Demarest insists that the community work benefits research, noting that the local Maya played a role in his most recent major discovery: the massacre of what he suspects was the royal court at Cancuén. Demarest's team found 34 bodies dressed in full regalia, including one with a necklace containing 36 jaguar canines. The locals helped recover looted monuments with glyphs that revealed the date that the victims were killed—800 C.E.—and that one of them was Kan Maax, Cancuén's king. Demarest considers the massacre to be one of the early signs of the collapse of Maya civilization, exhibiting a new, more "destructive" warfare; previous conquerors tended to take over cities rather than destroy them, he says.

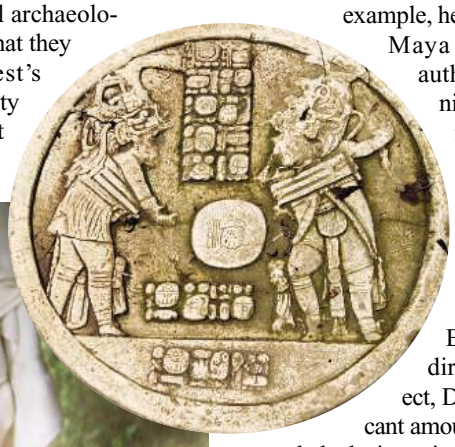
To fund such projects, Demarest has won major grants from a variety of sources, many of them outside the traditional research agencies. He has raised more than $5 million during the course of the Cancuén project, much of which goes to community-development endeavors managed by the local Maya. He says the U.S. Agency for International Development has awarded more than $1 million, and a National Institutes of Health matching grant has provided more than $600,000. Corporate and private donations have exceeded $300,000, he says.

A starring role

Demarest also gets plenty of publicity—his discoveries have been covered by *The New York Times*, *The Washington Post*, National Public Radio, the *News Hour with Jim Lehrer*, and other international media—and he seems to relish the spotlight.

Despite the high-profile discoveries, other researchers are quick to point out that Demarest is only one of many leading Mayanists. Chase says he respects Demarest's work but that no single archaeologist is shaping Maya research. Several archaeologists were critical of what they perceived as Demarest's publicity-seeking activity but declined to comment on the record. "He does it for his own aggrandizement," says one distinguished Maya archaeologist.





At work. At Cancuén, Arthur Demarest has excavated a royal palace and artifacts including a circular altar stone (*inset*) that was looted, then recovered with the help of locals.

The discovery that may have garnered the most attention was the treasure-laden tomb of the second great king of Petexbatún. Demarest says Touchstone, the movie production company, approached him about making a movie based on the 1991 field season, which took place during a civil war that ended in 1996, and he's writing a popular book about it. A movie about his work was produced by National Geographic, which has also served as one of his major patrons. However, during his SAA presentation Demarest harshly criticized the movie as simplistic. After meeting a National Geographic representative who attended the talk, he lamented his candor. "Another foot in my mouth. … I'm half Cajun and half Italian, and I can't control my mouth or my hands," he said, referring to his tendency to gesture.

Barbara Moffet, director of communications at National Geographic, says Demarest has been awarded an unusually high number of the organization's modest grants. "We have funded Dr. Demarest so extensively because he does dynamic, important archaeology," Moffet says. Working with Demarest, she adds, "is never dull."

Excitement, for Demarest, isn't defined exclusively by major discoveries. In 2003, for example, he, his crew, and the local Maya helped Guatemalan authorities recover a magnificent Maya altar taken from Cancuén by looters who were involved with drug traffickers, an event that received extensive media coverage. According to archaeologist Tomás Barrientos, who co-directs the Cancuén project, Demarest spent a significant amount of his own money to help the investigation. There were rumors that the criminals threatened to kill the archaeologists for testifying against them, and for a time Demarest hired bodyguards.

Unlike most American archaeologists working in Guatemala, Demarest lives there most of the year. Although he's on campus at Vanderbilt now, for much of the past 3 years he has been in Guatemala on extended leave. He raised two sons in Guatemala and recently married his second wife, a Guatemalan woman who shares his spartan existence when he is at Cancuén, which is hidden away in the jungle. His lifestyle is "very hard on your personal life," he says. At the same time, he takes pleasure in the roar of the howler monkeys, tolerates the bugs, and is unfazed by cold showers. "I intend to keep digging until I'm dead."

He often chooses new sites for excavation and works there "6 or 7 years, [to] produce a generation of students," he says. Eight of Guatemala's 10 Ph.D. archaeologists studied under him, and he believes that he has had far more impact on his Guatemalan than his U.S. students. "I don't feel like I'm very useful in the States," where he finds life "a little bit boring," he says. "You can do a world of good in Guatemala."

–MICHAEL BAWAYA

Michael Bawaya is the editor of *American Archaeology.*

CREDITS: J. RABINOWITZ PHOTOGRAPHY

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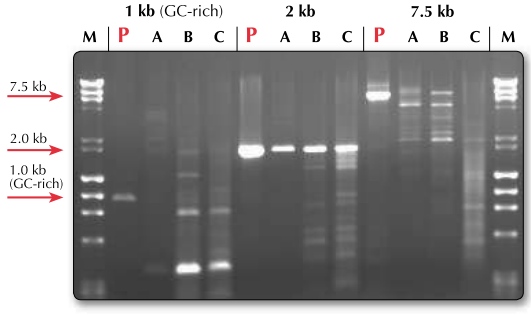

The following fragments were amplified from human genomic DNA: 1kb from CEBPB gene (GC-rich), 2 kb and 7.5 kb from human β-globin gene. Hot start high-fidelity DNA polymerases (P, A,B,C) were used according to suppliers' recommendations:

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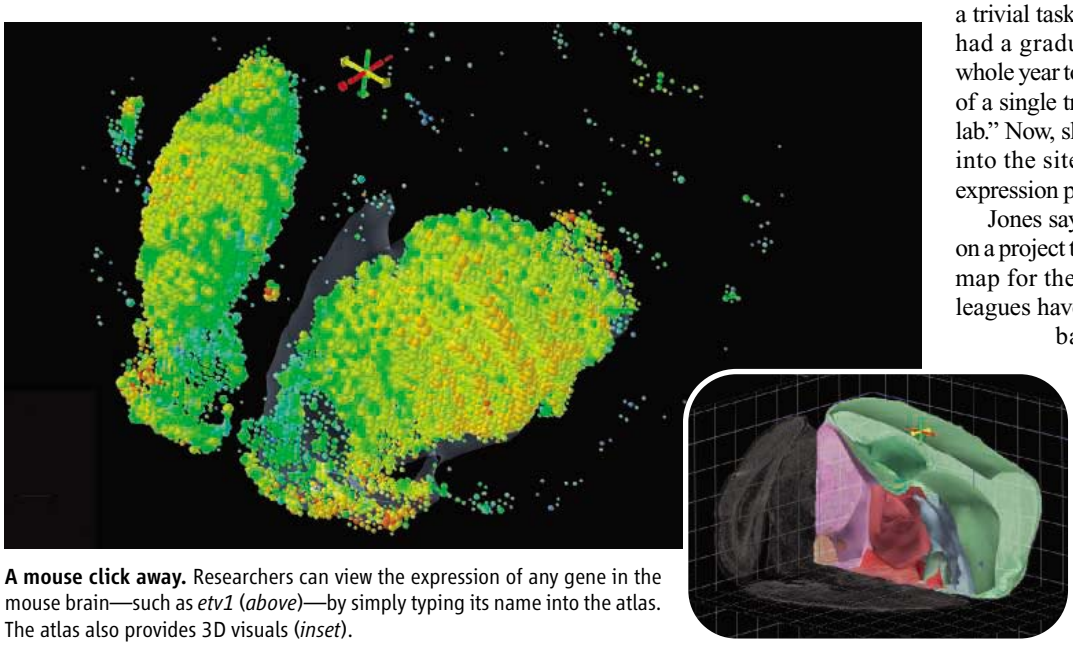
NEUROSCIENCE

'Google of the Brain': Atlas Maps Brain's Genetic Activity

With his Microsoft money, Paul Allen has funded a gene-expression database of the mouse brain

In 2002, Microsoft co-founder and philanthropist Paul Allen asked a handful of neuroscientists how best he could use some of his fortune to advance their field. The researchers recommended the creation of a map of gene expression in the mouse brain, which they said would combine with knowledge gained from the Human Genome Project to illuminate how our brains work. The idea became the inaugural project of the Allen Institute for Brain Science in Seattle, Washington; $40 million and 4 years later, the map is now finished.



A mouse click away. Researchers can view the expression of any gene in the mouse brain—such as *etv1* (*above*)—by simply typing its name into the atlas. The atlas also provides 3D visuals (*inset*).

CREDITS: ALLEN INSTITUTE FOR BRAIN SCIENCE

The completion of the Allen Brain Atlas, announced this week, caps a painstaking effort that involved analyzing more than 250,000 slices of mouse brain to determine which of the 21,000 or so known genes in the animal's genome are turned on in the brain, as well as where and to what extent. The result is a freely accessible, searchable digital database (www.brain-map.org). Researchers are ecstatic: They say the gene-expression map will accelerate the search for drugs to treat psychiatric illnesses and help address fundamental questions about the development and function of different brain structures. "Some are calling it the Google of the brain," says Joanne Wang, a pharmaceutics researcher at the University of Washington, Seattle. "I could not think of a better term than that."

The interdisciplinary project involved neuroscientists and geneticists, as well as bioinformaticists and software engineers who worked on automating the analysis. "It took more than a year just to integrate the microscope to the software," says Allan Jones, the chief scientific officer of the project. Having mice as the object of study allowed for a high degree of experimental control, Jones notes; not only were the animals genetically identical, but they were also given the same diet and handled the same way to the same age—56 days—before brain tissue was drawn from them.

The map shows that 80% of genes in the mouse genome are expressed in the brain—higher than the 70% figure that researchers previously thought. "Also, roughly 25% of the genes expressed in the brain show some kind of regional restriction," Jones says. Among the things that the data set will allow researchers to do, he adds, is "group cells in the brain that have similar patterns of gene expression, which could reveal functionally relevant brain structures that are still unknown."

The atlas, whose data have been made available in installments since December 2004, is already saving researchers a lot of time and trouble, says Jane Roskams of the University of British Columbia in Vancouver, Canada. Roskams, who studies the development of neurons in the vertebrate embryo, has been using the map to test how combinations of glutamate receptors on neurons in the olfactory bulb may be responsible for differences in how likely they are to undergo programmed cell death. The map helps narrow down alternative hypotheses quickly instead of testing them all through hours of lab work, says Roskams.

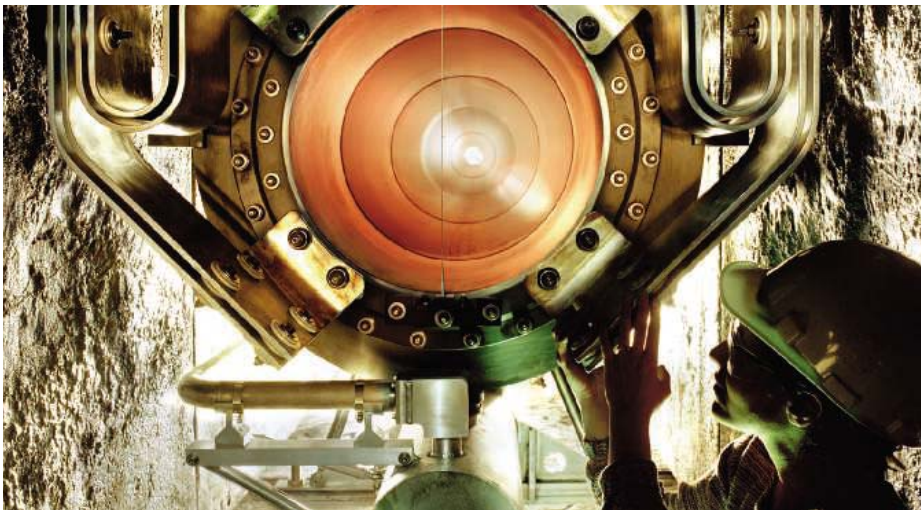
Wang has found that the atlas aids her work on using molecules known as membrane transporters, which ferry cargo into and out of the brain, to deliver drugs to targeted brain areas. Doing this requires understanding where and how abundantly drug-transporter genes are expressed in the brain, which is "not a trivial task for individual labs," she says. "I had a graduate student work for almost a whole year to map the brain expression pattern of a single transporter gene discovered in our lab." Now, she can simply type a gene's name into the site and click a button to view its expression profile.

Jones says the institute is now embarking on a project to create a similar gene-expression map for the human cortex; he and his colleagues have already begun talks with brain banks and neurosurgeons. "It's going to be more of an experimental venture," says Jones, "because we will have no control over the genetic background and the environmental factors behind the samples we end up analyzing."

Still, he believes the new project will provide important insights into genetic drivers of the overall structure of the cortex as well as how developmental abnormalities unfold. For example, Karen Berman, a psychiatrist at the National Institute Mental of Health in Bethesda, Maryland, plans to use the current mouse atlas as well as the planned human cortex map to home in on the specific gene defects that cause Williams syndrome, a developmental disorder she's studied for several years.

"This will also help us to look at interactions between genes of interest and how their effects develop in the brain over time," she says. "That could help us intervene before the disorder sets in." Researchers say the atlas will definitely quicken the development of such interventions and prove that Allen spent his money wisely.

–YUDHIJIT BHATTACHARJEE



Switcheroo. Neutrinos from this beamline in Illinois change type in transit to Minnesota.

Neutrino Physics Probes Mysteries Of 'Flavor' and Origins

Fifty years ago, Fred Reines and collaborators reported (in *Science*) the first confirmed detection of the elusive neutrino. At the meeting,* physicists marked the anniversary with a flood of new and anticipated neutrino data offering insights into particle physics and astronomy.

Neutrinos are produced abundantly throughout the cosmos. They are generated by cosmic rays striking Earth's atmosphere, by nuclear fusion reactions in the cores of stars, and in supernova explosions and other energetic cosmic processes. There are three known varieties, or "flavors," of neutrinos, associated with the electron, muon, and tau particles (collectively known as leptons).

As they propagate through space, neutrinos travel not as pure flavors but rather as mixtures, sort of like a vanilla-chocolate swirl. Neutrinos beginning a journey as primarily one flavor may end up being detected as another. Such identity switches, or "flavor oscillations," have been confirmed by several neutrino experiments, most recently by an international collaboration known as MINOS.

The MINOS detector, housed in an iron mine in Soudan, Minnesota, measures neutrinos produced at the Fermi National Accelerator Laboratory, 735 kilometers away in Batavia, Illinois. The latest results confirm the disappearance of muon neutrinos in transit, suggesting a switch of flavors on the way, said Donna Naples of the University of Pittsburgh in Pennsylvania, reporting at PASCOS for the collaboration.

"We see a significant deficit" of muon neutrinos, Naples said. The Soudan detector recorded 215 events in the relevant energy range, compared with more than 300 expected if the neutrinos did not shift flavors. The collaboration's paper with the new data has been submitted for publication and is available online at arxiv.org/abs/hep-ex/0607088. Further data, Naples said, will help pin down neutrino properties more precisely, in particular the masses of the three neutrino types and the precise amount of mixing between the flavors.

For astronomy, much of the current neutrino excitement focuses on understanding the more energetic neutrinos that may shed light on the nature of high-energy gamma rays in space and the origin of cosmic rays. "Cosmic rays are totally not understood. We don't know where they come from or how they are accelerated," said neutrino physicist Francis Halzen of the University of Wisconsin, Madison.

Because high-energy neutrinos may be produced in the same processes that create cosmic rays, several experiments are now planned or in progress to seek neutrinos at the top of the energy scale, exceeding 1 trillion electron volts (TeV). Hopes are highest for results from IceCube, a massive detector array partially completed more than a kilometer below the ice's surface at the South Pole.

IceCube detects neutrinos produced when muons from cosmic ray showers collide with atoms in the ice and may also be able to measure the arrival of TeV neutrinos from space. If such neutrinos are found, they could help identify the mechanism that produces high-energy gamma rays, reported Matt Kistler of Ohio State University, Columbus. Recent observations from observatories such as HESS in Namibia have revealed the existence of extremely high-energy gamma ray sources in the galaxy. It's unclear whether these high-energy rays are produced by a process involving leptons (electrons colliding with photons) or hadrons (the decay of pions). If pion decay is the cause, high-energy neutrinos would also be produced, and their detection by IceCube or other neutrino experiments could verify the pion-decay explanation, Kistler said.

In any case, neutrino experiments now operating, under construction, or planned promise to turn what was once a hypothetical particle into a powerful astronomical tool—a prospect that Reines himself envisioned at the time of his experimental discovery, Halzen said.

"People at the time didn't know whether [the neutrino] was a mathematical trick to fix up theory or whether it was a real particle," Halzen said. "As soon as Reines discovered a real particle, he suggested that this was a way to do astronomy."

* 12th International Symposium on Particles, Strings, and Cosmology.

A Cosmic-Scale Test for String Theory?

Theorists who think nature's ultimate building blocks are vibrating strands of energy called superstrings have always had a big problem converting skeptics. One reason: The strings are far too small to see. In fact, they are supposedly so small that no conceivable microscope (or particle accelerator) could ever render them visible. But some string theorists now believe they've found a way to make superstrings observable: supersize them.

Superstrings that were supertiny shortly after the big bang could have been stretched by the expansion of the universe to cosmic size today, Robert Myers of the Perimeter Institute for Theoretical Physics in Waterloo, Ontario, Canada, noted at the meeting. He

CREDIT: PETER GINTER

described several ongoing investigations of the properties that such cosmic strings would have and how they might be detected.

"I think it's pretty exciting," said Princeton University astrophysicist David Spergel. "It's the potential to see physics from really high energies that we can't get any other way. It's the potential for a really exciting surprise."

Earlier analyses indicated that strings from the time of the universe's birth would be diluted away by the subsequent expansion or would be too unstable to survive to the current epoch. But advances in recent years have shown that's not necessarily true, Myers said. "We have scenarios where you get, in fact, a rich network of different kinds of strings generated at exit from inflation," the brief burst of superfast expansion following the big bang, Myers said. "So we have the exciting possibility that in certain scenarios, the superstrings might appear in a network of cosmic strings."

One way of detecting cosmic superstrings would rely on precise timing of the spinning rates of fast pulsars, neutron stars that emit blips of radiation at precise intervals. Small variations in the intervals might be the effect of background gravitational waves produced by cosmic string networks.

Another promising approach, Myers said, would be to detect gravitational waves directly by means of experiments such as LIGO, the twin observatories in Louisiana and Washington state (*Science*, 21 April 2000, p. 420). As cosmic strings wiggle, rapid acceleration of their mass can generate powerful gravitational-wave beams. LIGO may not be sensitive enough to detect them, but a planned set of three space-based gravitational wave detectors known as LISA would be a good bet.

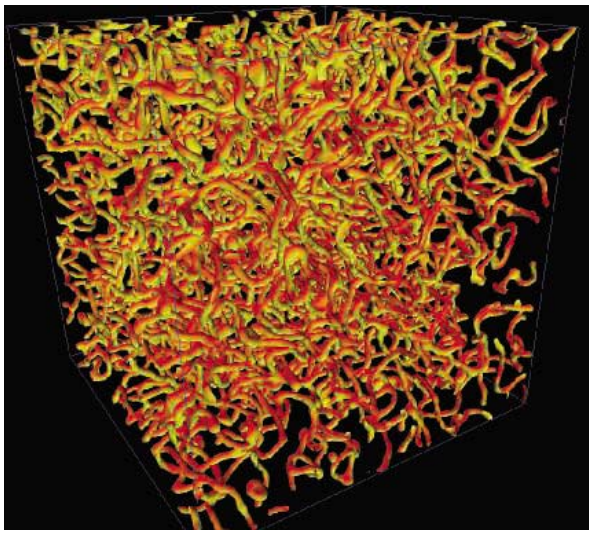
Another method would exploit gravitational lensing, the power of massive objects to bend light passing nearby. The gravity of a string in space could bend light enough to split the image of a galaxy in the background, giving astronomers the impression of two identical galaxies sitting side by side.

Finally, physicists might detect distortions imprinted by strings in the cosmic background radiation, the smooth glow of microwaves from the big bang that permeates all of space. Evidence could come from extremely precise measurements such as those expected from the European Space Agency's Planck Surveyor mission, scheduled for launch next year.

"We have people actually working on looking for string signatures for their thesis," said Spergel. "So maybe we'll detect them."

–TOM SIEGFRIED

Tom Siegfried is a writer in Los Angeles, California.



Pumped up. Computer simulations show that cosmic inflation might have created networks of enormous "superstrings."

Snapshots From the Meeting >>

Shooting the moon. Recent observations of a galactic cluster collision seem to rule out modified theories of gravity for explaining dark matter (*Science*, 25 August, p. 1033). But Gia Dvali of New York University reports that other changes to the law of gravity over vast distances are still possible and could explain why the universe appears to be expanding at an accelerating rate.

In fact, such modifications would produce observable variations in the orbit of the moon around Earth, Dvali pointed out. Deviations on the order of 1 millimeter would be a sign that mysterious "dark energy" is not needed to explain cosmic acceleration. Such precise measurements may be within the reach of a new generation of laser-ranging experiments, in which researchers bounce laser beams off reflectors that Apollo astronauts left on the moon.

"It would be absolutely amazing," Dvali said. "By looking at the moon, you can derive information about dark energy."

Dark possiblities. In the cosmos, invisible, unidentified "dark" matter outmasses the ordinary "baryonic" matter known on Earth about 10 to 1. That may sound like a big difference, but to physicists it's mystifyingly close. It suggests that dark matter and ordinary matter were originally produced by related mechanisms.

At the meeting, physicist Leszek Roszkowski of the University of Sheffield in the U.K. discussed the possibility that both forms of matter owe their origin to Q balls, exotic objects possibly formed in the early universe. A Q ball is basically a bag of squarks, hypothetical partner particles to ordinary quarks predicted by a theory known as supersymmetry. If Q balls decayed into both ordinary matter and dark matter, it would explain why the amounts of the two forms of matter are similar.

That idea doesn't work if the dark matter is composed of WIMPs: weakly interacting massive particles that supersymmetry also predicts. Accelerator experiments and searches for dark matter using underground detectors rule out the mass range for WIMPs predicted for Q-ball decay. But Roszkowski and collaborator Osamu Seto of the University of Sussex, U.K., calculate that those objections to the Q-ball scenario can be avoided if Q-ball decay produces another supersymmetric particle called the axino. If so, the axino's mass would be about the same as the proton's—much less than the mass predicted for WIMPs and not detectable by current underground experiments.

"That's bad news for WIMP dark-matter searches," said Roszkowski. But it's possible that evidence for axinos could be produced in the Large Hadron Collider, scheduled to begin operation outside Geneva next year. **–T.S.**

CREDITS (TOP TO BOTTOM): PAUL SHELLARD/UNIVERSITY OF CAMBRIDGE; USGS/JPL/NASA

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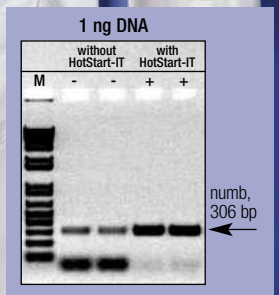

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EDITED BY YUDHIJIT BHATTACHARJEE

Three Q's >>

University of Ottawa historian **Chad Gaffield**, 55, last week set aside his duties at the helm of Canada's best funded social sciences project—an initiative that makes census data available for research purposes—to become the president of the government's troubled $272 million Social Sciences and Humanities Research Council (SSHRC).

Q: The consensus is that SSHRC is in disarray. Your predecessor's strategic planning exercise was botched. There's a raft of vacancies on the governing board and within the agency as staff keep bailing. How will you clean up the mess?

By completing the team within SSHRC, getting shoulder to shoulder with the research community, and enhancing our contributions—and the perception of them—to Canadian society and the international community.

Q: SSHRC needs political and private sector allies. Do you have any mechanisms in mind to develop those?

We are an agency focused on people, and it's in the interests of all businesses, governments, and institutions to enhance human assets. One of the ways for SSHRC to link to the community is through our governance structure, by getting broader representation on the 22-member SSHRC council.

Q: Why move from eminent historian to whipping boy?

It's a way to contribute to one of the most pressing and important challenges facing Canadians and the world, which is how individuals and groups can live together for everyone's benefit.

Got a tip for this page? E-mail people@aaas.org

CREDITS (TOP TO BOTTOM): MARTIN LIPMAN; STEPHNE JOHNSON; JESPER MADSEN

PIONEERS

SOCIAL ENTREPRENEUR. Jim Fruchterman applies technology to improve people's lives through the nonprofit Benetech Initiative he founded in 2000. Thanks to the John D. and Catherine T. MacArthur Foundation, he hopes to do even more over the next 5 years.

Last week, the foundation named him one of 25 MacArthur Fellows. The 47-year-old engineer plans to use the $500,000 prize to write a book that "will inspire the technology and science and business communities to see that technology benefits all of humanity, not just the richest 10%."

As a student, Fruchterman designed a reading machine for the blind. Current projects at Benetech, based in Palo Alto, California, include a computer database for human-rights activists and an inexpensive land-mine detector. This year's class of fellows also includes Victoria Hale, a San Francisco pharmaceutical chemist and entrepreneur who's developing drugs for neglected diseases, and Lisa Curran, a tropical biologist at Yale working on strategies to fight deforestation. See the complete list at www.macfound.org.

AWARDS

INNOVATORS. Thirteen biomedical researchers have been chosen to receive the 2006 Pioneer Awards: $2.5 million, 5-year grants from the National Institutes of Health (NIH) for researchers doing highly innovative science. Four winners this year are women, compared to six of 13 last year and none in the inaugural 2004 class; their absence sparked a protest that led NIH to revamp the awards process (*Science*, 22 October 2004, p. 595). The list is at nihroadmap.nih.gov/pioneer/Recipients06.aspx.

HEINZ PRIZES. A pioneer of systems biology and a founder of the green chemistry movement have won $250,000 awards from the Heinz Foundation.

Leroy Hood, founder of the Institute for Systems Biology in Seattle, Washington, is being honored for his role in developing the DNA sequencer used to spell out the human genome and other key biotechnical instruments. And Paul Anastas, who worked at the U.S. Environmental Protection Agency in the 1990s, is being recognized for encouraging companies to adopt environmentally benign chemical processes. The full list of Heinz award winners is at www.heinzawards.net.

Campaigns >>

COOL IT. Since 2004, David Shearer has enjoyed yearly visits to the Burning Man festival, which draws more than 35,000 revelers, artists, and anarchist tent-dwellers to Nevada's Black Rock Desert in early September. But last year, the epidemiologist-turned–environmental consultant decided to take the event's Leave No Trace principle one step further.

He calculated the amount of carbon dioxide emitted from the actual burning of the man statue, a yearly highlight. The growth, transport, and burning of the wood in the 38-ton statue, Shearer found, produced 110 tons of equivalent carbon emissions. To offset those emissions, he and a colleague, Jeff Cole, began raising money for renewable-energy projects such as methane capture in Pennsylvania and wind farming in South Dakota. Their site, coolingman.org, has topped its initial goal of $1200.

Now, Shearer is encouraging participants to purchase similar credits to offset travel to the festival, onsite energy use, and the ubiquitous fire art that pervades the festivities. "I'm trying to rebrand the idea of being cool," he says.

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LETTERS

edited by Etta Kavanagh

A Fresh Look at Innovation and Security

INNOVATION IS THE LIFEBLOOD OF U.S. COMPETITIVENESS AND SECURITY. U.S. BUSINESSES and universities must be able to tap the world's best scientific minds to fuel innovation, while preventing sensitive knowledge from falling into the hands of those who would do us harm. The Bush Administration has begun taking a fresh look at U.S. policies affecting the transfer of sensitive U.S. technology to foreign nationals, also known as "deemed exports," to ensure that these policies advance U.S. security and prosperity.

The same expanding flow of people, products, capital, and information across national borders that has catapulted productivity and innovation has also created new threats. Thus, it is important to pursue the complementary objectives of ensuring access for and implementing effective restrictions on foreign nationals working in U.S. companies and research universities. Almost 20% of tenured science and technology faculty are foreign-born, and nearly 60% of postdoctoral scholars in science and engineering are working here on a temporary basis (*1*). This dependence is likely to increase.

"[P]olicies intended to protect these technologies from misuse **could have the opposite effect…**"
—McCormick

At the same time, the openness of the U.S. research community also brings with it risks that sensitive technology will be obtained by nations or individuals who threaten our security. As reported by the 2005 National Counterintelligence strategy, "More than 90 countries target sensitive U.S. technologies" [(*2*), p. 5].

Some believe that foreign access to U.S. technological breakthroughs must be sharply restricted, while others maintain that U.S. technological leadership requires minimizing controls on the transfer of sensitive knowledge to foreign researchers. The Administration, led by Secretary of Commerce Carlos Gutierrez, has demonstrated a firm commitment to achieving the difficult but essential equilibrium that this issue requires. Several key principles guide our efforts:

1) The world is changing too rapidly for our policies to be confined by historical precedents or dated viewpoints regarding current regulation of deemed exports.

2) The transfer of sensitive technology is not, as some believe, a zero-sum trade-off between innovation and security. For example, few would dispute the importance to U.S. security of leadership in advanced composites, such as material used in the manufacture of commercial aircraft, or thermal imaging. Yet, if we are not careful, policies intended to protect these technologies from misuse could have the opposite effect of reducing U.S. research, production, and investment in these areas.

3) If we are to achieve our joint goals of security and competitiveness, future restrictions on technology transfers must be targeted only at the people, technologies, and activities that pose the most potent risks. Regulations too broad in scope will ultimately undermine both goals by spreading limited resources, focus, and leadership attention too thinly.

4) The U.S. R&D community must be the front line of defense in mitigating security threats. An effective approach must rely on the support and active participation of those conceiving and conducting sensitive research.

Secretary Gutierrez has taken on the challenge of reforming deemed export policy by establishing a Deemed Export Advisory Committee (*3*), co-chaired by former Lockheed Martin Chairman and CEO Norman Augustine and former CIA Director and President of Texas A&M University Robert Gates. The Committee will undertake a comprehensive policy review and make recommendations on the appropriate scope and direction of deemed export controls. This very distinguished independent group will have the cooperation of key departments throughout the government, access to related intelligence reporting, and a dedicated staff to support its efforts. Within 12 months, it will provide recommendations to Secretary Gutierrez regarding the future of this important policy.

DAVID MCCORMICK

Under Secretary of Commerce for Industry and Security from 2005 to 2006, and currently White House Deputy National Security Advisor for International Economic Affairs, The White House, National Security Council, Washington, DC 20504, USA.

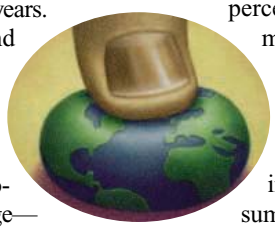
References and Notes

1. 2005 National Intelligence Council Report (National Intelligence Council, Washington, DC, 2005).
2. 2005 National Counterintelligence Strategy (Office of the National Counterintelligence Executive, Washington, DC, 2005).
3. Committee members: Albert Carnesale, former Chancellor of the University of California at Los Angeles; Ruth David, President and CEO, Analytic Services, Inc.; John Engler, President, National Association of Manufacturers; Anthony Frank, Provost and Senior Vice President, Colorado State University; General John A. Gordon, former Deputy Director, Central Intelligence Agency; Sean O'Keefe, Chancellor, Louisiana State University; Eva Pell, Senior Vice President and Dean of the Graduate School, Penn State University; Michael Splinter, CEO, Applied Materials; James Siedow, Vice Provost for Research and Professor of Biology, Duke University; William A. Wulf, President, National Academy of Engineering, and Professor of Computer Science, University of Virginia.

Does the World Really Need More Babies?

TO SEE AN ARTICLE LIKE "THE BABY DEFICIT" (M. Balter, Special Section: Life Cycles, News, 30 June, p. 1894) in a magazine such as *Business Week*, or *The Economist*, or *Sociology Today* would not be surprising, but how could *Science* publish such an article? The thrust of this four-page article is captured in the sentence "Population losses could bring a raft of negative economic consequences in the industrialized world, as well as greater stresses on social security and health care systems as the proportion of older citizens increases."

The word "environment" never once appears in the article. With the biosphere on the brink of multiple disasters or even catastrophe, how can *Science*—the publisher of numerous reports on energy, climate change, and species extinction—write so extensively about "baby deficits" without mentioning overpopulation in an environmental context? Our species is presently responsible for the worst mass extinction event of the past 65 million years. If recent reports of imminent and rapid destruction of the entire Amazon rainforest—due to activities like logging, burning, and agriculture accompanied by prolonged drought from anthropogenically induced climate change—turn out to be accurate, then our species may ultimately become responsible for the worst mass extinction in the entire history of life on Earth. Viewed in this light, a rapid and massive decline in human numbers due to a "baby deficit," although perhaps accompanied by some short-term economic and social pain for some people, would be a blessing for millions of species on Earth, including our own.



BEN ZUCKERMAN

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Balancing Fertility Rates with Resources

MICHAEL BALTER'S ARTICLE "THE BABY DEFICIT" (Special Section: Life Cycles, News, 30 June, p. 1894) overlooks a yawning chasm in perception. As Balter reports, economists concerned with economic growth view low fertility in developed countries with alarm. At the same time, ecologists grimly document the global impact of excessive resource consumption in the same countries.

Moreover, total fertility rates (TFRs) alone do not describe the trajectory of global population and its impact. China has a below-replacement TFR (1.6), but its still-growing population combined with economic growth is creating horrendous environmental and health impacts, as well as stressing global markets for raw materials.

Human consumption already exceeds the carrying capacity of the planet by 23% (*1*). In the foreseeable future, global climate change and collapsing biodiversity may substantially reduce Earth's carrying capacity. The looming peak in global oil production, undermining the heavy energy subsidy it provides to agriculture, could shrivel the Green Revolution and exacerbate the shortfall.

Since the start of the industrial age, the human population and the economy that supports it have experienced exhilarating growth. As with a narcotic, the initial sense of well-being has given way to addiction and destruction.

We view falling TFRs as a welcome sign that the human population is undergoing detoxification from its addictive growth. The question is whether the economic system can weather the stress of withdrawal. Continued efforts to accelerate the demographic transition in developing countries are imperative. Equally imperative are creative economic thought and political effort that focus not on maintaining impossible exponential growth of consumption, but on the steady supply across the globe of the critical goods and services that humans need to lead lives of happiness and dignity.

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*Author of a monthly column, entitled "Population Matters," for the *Durango Herald*.

Reference

1. See www.ecofoot.net/ (accessed 4 Aug. 2006).

CORRECTIONS AND CLARIFICATIONS

Perspectives: "Waterborne infectious diseases—could they be consigned to history?" by A. Fenwick (25 Aug., p. 1077). Editing resulted in two misleading sentences. The final sentence in the Schistosomiasis section (p. 1080) should have read, "In Uganda, three rounds of treatment with praziquantel and albendazole reduced the prevalence of the *S. mansoni* infection by more than 50% and the intensity by 90%, as well as reducing the intensity of hookworm by 95% (*25*)." The third sentence in the Malaria section (p. 1080) should have read, "Control of mosquitoes is difficult and the malaria parasite is capable of developing resistance to successive generations of antimalarial drugs (*26*)."

Reports: "A dielectric polymer with high electric energy density and fast discharge speed" by B. Chu *et al.* (21 July, p. 334). The affiliations were incorrect. They should appear as follows:

Baojin Chu,[1] Xin Zhou,[2] Kailiang Ren,[2] Bret Neese,[1,3] Minren Lin,[1] Qing Wang,[1,3] F. Bauer,[4] Q. M. Zhang[1,2,3*]

[1]Materials Research Institute, [2]Electrical Engineering Department, [3]Materials Science and Engineering Department, Pennsylvania State University, University Park, PA 16802, USA. [4]Institute Franco-Allemand de Recherches, 5 Rue du General Cassagnou, 68300 Saint-Louis, France.

Reports: "Electric fields at the active site of an enzyme: direct comparison of experiment with theory" by I. T. Suydam *et al.* (14 July, p. 200). An atom-labeling error occurred in the final preparation of Fig. 1C. The IDD inhibitors should be drawn as phenoxyacetic acids, not carbonic acids. See M. C. Van Zandt *et al.* [*Bioorg. Med. Chem.* **12**, 5661 (2004)] for the correct structures.

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Asymmetric Coevolutionary Networks Facilitate Biodiversity Maintenance"

J. Nathaniel Holland, Toshinori Okuyama, Donald L. DeAngelis

Bascompte *et al.* (Reports, 21 April 2006, p. 431) used network asymmetries to explain mathematical conditions necessary for stability in historic models of mutualism. The Lotka-Volterra equations they used artificially created conditions in which some factor, such as asymmetric interaction strengths, is necessary for community coexistence. We show that a more realistic model incorporating nonlinear functional responses requires no such condition and is consistent with their data.

Full text at www.sciencemag.org/cgi/content/full/313/5795/1887b

RESPONSE TO COMMENT ON "Asymmetric Coevolutionary Networks Facilitate Biodiversity Maintenance"

Jordi Bascompte, Pedro Jordano, Jens M. Olesen

Mutualistic networks are characterized by weak and asymmetric interactions, which a simple model predicts will facilitate species coexistence. Holland *et al.* propose a more complex model and argue that coexistence is independent of mutualism strength. However, we show that mutualism strength still plays an important role in their model and that it significantly decreases with species richness as predicted.

Full text at www.sciencemag.org/cgi/content/full/313/5795/1887c

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

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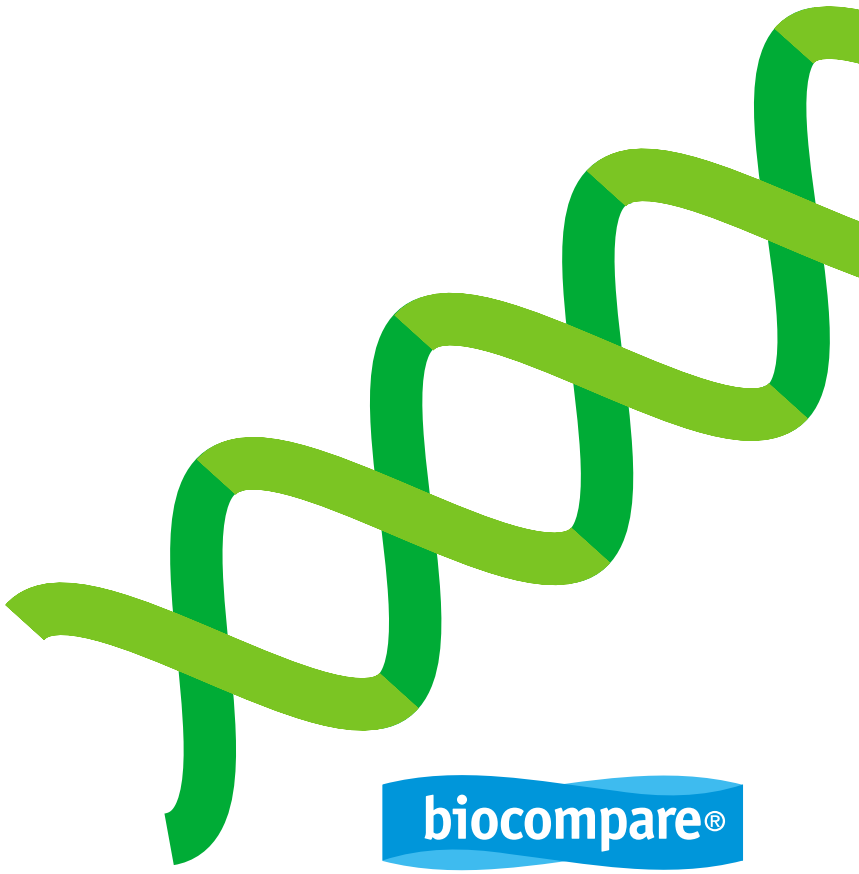

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MEDICINE

The Politics of Hope—Dreaming in a Genomic Age

Sandra Soo-Jin Lee

The completion of the Human Genome Project leaves us wondering what comes next. In spite of substantial strides toward efficiency and economy in identifying functionally pertinent human genetic variation, the question remains: What, if anything, will developments in human genetics mean for improved human health and well-being? And will the utopia of personalized medicine change the lives of all groups equally? Despite predictions of an imminent era of genomic medicine where race as a genetic proxy will be rendered obsolete, the struggle over how to interpret the meaning of genetic differences among groups persists. The much-heralded mantra of the post–Human Genome Project age that race is not genetic continues to be a controversial and highly contested claim.

The Troubled Dream of Genetic Medicine, a concise and well-argued book, illuminates the enduring salience of race for genetics and human health. In a field with few accounts of the social and cultural imbrication of genomic science and biomedicine, historians Keith Wailoo (Rutgers University) and Stephen Pemberton (New Jersey Institute of Technology) offer a complex, comparative analysis of three diseases: Tay-Sachs, cystic fibrosis, and sickle cell. The authors argue that the trajectory of research and the ultimate management of these diseases are deeply influenced by conventional ideas about racial difference and by local politics that are inextricable from questions about social identity.

A central theme of the book is the resilience among scientists, clinicians, and patients of their belief in the promises of medical innovation despite histories that might dictate caution. The authors argue that such privileging of therapeutic hope is particularly potent in the context of emerging genomic technologies, where the allure of technical solutions fuels indefatigable faith in "cutting-edge" science. However, as Wailoo and Pemberton suggest, these strides toward fulfilling the promises of genomic medicine follow negotiated paths that are often pockmarked by the ongoing struggle over the meaning of racialized difference. Citing the work of Herbert Gans on symbolic ethnicity (*1*), Wailoo and Pemberton suggest that although naturally bounded communities may no longer exist, racially and ethnically organized groups are emerging around powerful symbols, such as the iconic gene.

The close characterization of Tay-Sachs disease with Jewish identity resulted in a highly orchestrated effort within Jewish communities to use genetic screening in marital and reproductive choices. Efforts at identifying Tay-Sachs carriers advocated by orthodox Jewish organizations such as Dor Yeshorim became enmeshed in rhetoric around "so-called Jewish genes" and Jewish self-determination and self-preservation. The expansion of endorsed genetic testing for Tay-Sachs to other less-lethal diseases such as Gaucher's put in stark relief how diseases "become Jewish" and the slippery slope upon which preventive health campaigns for genetic testing can descend.

The Troubled Dream of Genetic Medicine
Ethnicity and Innovation in Tay-Sachs, Cystic Fibrosis, and Sickle Cell Disease

by Keith Wailoo and Stephen Pemberton

Johns Hopkins University Press, Baltimore, MD, 2006. 259 pp. $60, £40. ISBN 0-8018-8325-3. Paper, $21.95, £14.50. ISBN 0-8018-8326-1.

Although cystic fibrosis has been identified as a "Caucasian disease," the authors suggest it was comparatively less circumscribed by race. Rather than being discussed with an emphasis on the hereditary nature of the disease, cystic fibrosis has been described in terms of its biochemical abnormalities and recast as a debilitating lung disease that can and does affect all populations. This racial effacement of cystic fibrosis may have been the result of scientists beginning to understand the political landscape of racialized diseases such as Tay-Sachs and sickle cell. Delimiting cystic fibrosis as a "white disease" would do little to increase limited resources for its research and management. Building on the relative invisibility of "whiteness," cystic fibrosis was represented by the ailing panethnic lung, which allowed a coalescence of hope and entrepreneurialism toward a more universal goal of therapeutic breakthroughs.

The opposite, the authors argue, was true of sickle cell disease, which (strongly associated with African Americans) has been deeply inscribed as a "black disease." In this third case, the authors present a powerful illustration of the complexity of racialization, amplifying Wailoo's highly acclaimed social history of the disease in Memphis, Tennessee (*2*). They describe how proposed cures and methods for management of sickle cell disease, such as bone marrow transplantation, must be interpreted within the context of previous exploitation of African Americans in biomedical research [such as the Tuskegee syphilis study sponsored by the U.S. Public Health Service (*3*)]. This legacy, coupled with increasing awareness of endemic disparities in access to healthcare, ensured that any discussion of therapeutic innovation was tethered to difficult discussions of risks, benefits, and social justice.

The book reveals the inherent moral economy in the interpretation, representation, and treatment of the three diseases. It places in stark relief the high stakes over how race is ultimately deployed in biomedical research. The disparate experiences of communities struggling with the promise of the genomic era make it plain that any discussion of race and genetics requires us to confront the inherent contradictions of how we assess meaningful difference. Genomic medicine demands that we grapple with the challenging questions put forth by the authors: "How heavily should we rely on the profit motive to shape the future of medicine and society? What level of risk should come with new medicines, and who should bear those risks? What limits should be placed on how individuals shape their genetic destiny?"

As the authors aptly illustrate, the answers to these questions are inevitably influenced by racial identification and the formation of racial categories. Although the authors demonstrate how these processes illuminate much of how communities negotiate the politics of hope in health, focusing solely on the workings of symbolic ethnicity may obscure other powerful fault lines such as gender, education, age, and class. Considering these may reveal different experiences and perspectives pertinent to the historical trajectories of the cases presented in the book. To be sure, race, as a correlative of biological difference, has set us on a defined path toward the as-yet illusory age of personalized medicine. We are nearing the creation of pharmacogenomic products tailored to particular groups identified in racial terms, as forecast by the authors—a development foreshadowed by the U.S. Food and Drug Administration's recent approval of BiDil, an

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anti-hypertension drug labeled for use exclusively on African-American patients.

Essential reading for anyone interested in genetics, disease, and the meaning of race, *The Troubled Dream of Genetic Medicine* is a notable contribution to the study of the intersection of science and society. Wailoo and Pemberton provide important historical context to the disparate courses of the three genetic diseases, emphasizing that the experience of disease is embedded in a social landscape that builds on prevailing values, attitudes, and beliefs. The powerful refraction of the prism of race in the detailed accounts of Tay-Sachs, cystic fibrosis, and sickle cell provides further evidence that science alone cannot render race obsolete. As long as race continues to be a salient dimension along which our society assigns meaningful social and biological differences, it will have an indelible influence on how we interpret health and disease.

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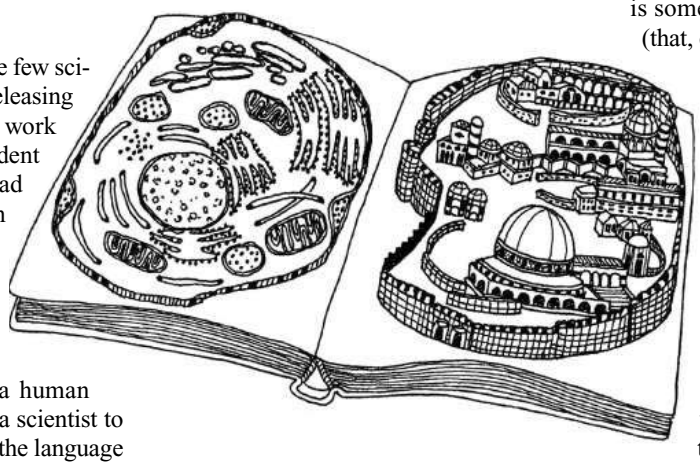
10.1126/science.1132060

SCIENCE AND RELIGION

DNA, Evolution, and the Moral Law

Robert Pollack



CREDIT: AMY POLLACK/FROM R. POLLACK, *THE BIOLOGY OF FAITH AND THE FAITH OF BIOLOGY* (COLUMBIA UNIV. PRESS, NEW YORK, 2000)

The Language of God
A Scientist Presents Evidence for Belief

by Francis S. Collins

Free Press (Simon and Schuster), New York, 2006. 303 pp. $26, C$32.95. ISBN 0-7432-8639-1.

Francis Collins is among the few scientists who can write of releasing the publication of his work while standing next to the president of the United States. As the head of the NIH's National Human Genome Research Institute, he stood in early 2000 with President Bill Clinton and Celera's Craig Venter to announce the completion of a draft sequence of the 3 billion base pairs of a human genome. It is almost as rare for a scientist to claim his work to be written in "the language of God," as he claims for this DNA sequence in his book by that title. He has written well for a general audience. To the best of my ability to judge, the facts of nature are laid out clearly. His religious life is as well, and that makes the book rare if not unique. But still, what can he mean by "the language of God"?

Midway through the book, Collins delivers a clear and cogent answer: He is an evangelical Christian. He sees no difficulty in accepting the continuity of life from its origins on Earth some 4 billion years ago. He makes plain that the continuity of life since then has depended upon the physical continuity of DNA backbones whose sequences undergo random mutation. He accepts wholeheartedly the complete capacity of natural selection to explain the emergence of new forms of life with new complexities, and of higher taxa emerging from long-lost species, over time. He sees humanity as but one example of that fecundity. And lastly, he sees all of this as being the intention of a Creator God, whose continued interest in Creation is exemplified not by any particular miracle but rather by these scientific facts.

Fair enough, but still, why does his particular expression of faith feature the human genome, or any DNA, in particular? The book's subtitle, *A Scientist Presents Evidence for Belief*, embodies his answer, a heartfelt if not entirely consistent one. The evidence for belief that he presents in the book comes down to the presence in himself and others of what he calls, after C. S. Lewis (*1*), the Moral Law. This is the apparently universal human propensity to ask of oneself "What ought I do?" and to decide that what one ought to do to others is what one would wish others to do to oneself, no more and no less. I happen to agree with this myself, and I too consider myself a religious person as well as a scientist.

But surely I would not want to make of this subjective emotional experience, however ubiquitous, evidence of the sort that a scientist marshals to confirm a hypothesis. Nor would I want to make the case, as Collins seems at first to be doing, that the Moral Law is somehow encoded in the human genome (that, certainly, is the simple meaning of his book's title and subtitle).

Despite the title he has chosen, the author knows and clearly states that the biology is otherwise: The human genome encodes the instructions for the assembly of what is after all a learning organism, not for what it then learns. Mental states are the product of social interaction from birth; in principle, any brain can have any thought. The Moral Law may well be God's presence among us—I do not know how to disprove this nor why one would try—but if so, it cannot be reduced to a DNA sequence, not even to the whole human genome.

But if the Moral Law were not written in DNA, then why would DNA be the "language of God" at all? In his credo "Science and Faith in Harmony," Collins explains. To see our species as embedded in a web of life and descended through natural selection from common ancestors with whom all life is shared is to see not human DNA but DNA per se as the prerequisite for the emergence of a life form capable of asking the question "What ought I do?" This least biological of questions is to him not then an example of an encoded voice. Rather, it is evidence of a heavenly plan launched by a caring God from a timeless place, a plan that has played itself out not only through all of life's DNA-encoded common ancestry but in the emergence of a universe capable of DNA-encoded life in the first place.

Taking biology as derived from the Greek words for life (*bios*) and word or knowledge (*logos*), he calls this personal religious vision of nature "BioLogos." Because the resonance of the slight change in spelling is with the Gospel of John—"In the beginning was the Word [Logos]"—he introduces a

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Christian particularity here that is not otherwise explicitly part of his religious vision.

Collins has done a brave thing in laying out his own religious convictions in a way that permits him to appeal to his fellow evangelical Christians to cease their war with nature and to accept the facts of life as discovered through science. By itself, this makes his account worth reading. But for one whose faith lies in meeting the Moral Law's demand, Collins reveals a couple of instances of diminished conviction that I find somewhat troubling. First, given that Collins makes so clear a case for the necessity of knowing the entire human genome (even though only one part in 70 encodes a protein), it is surprising to find that he has a much less stringent expectation for the texts that write of God directly: "Much of what I found in the CliffNotes versions of different religions (I found reading the actual sacred texts much too difficult) left me thoroughly mystified."

More substantial, as a person subject by his own conviction and faith to the Moral Law, surely Collins ought to have told us more about why James Watson vacated the position of director of the Human Genome Institute and why he then took it. He mentions that Watson left out of a conviction that the genome sequences should be a public good and not a gold mine of patents. But then the author fails to fill in the obvious gap: How did he keep within the Moral Law as he understands it when he stood with the head of Celera, the company that also announced its completion of a sequencing of the human genome, one that it hoped to profit from by patenting the interesting bits?

Collins refers to the parable of the Good Samaritan as an example of the Moral Law in action. Forty years ago, Martin Luther King, speaking at Riverside Church one year to the day before he was assassinated, extended the meaning of this foundational Christian text from the personal to the social:

> On the one hand, we are called to play the Good Samaritan on life's roadside, but that will be only an initial act. One day we must come to see that the whole Jericho Road must be transformed so that men and women will not be constantly beaten and robbed as they make their journey on life's highway. True compassion is more than flinging a coin to a beggar. It comes to see that an edifice which produces beggars needs restructuring.

The place of Moral Law in science thus becomes obvious: it is to oblige us all to ask, how can my science contribute to this restructuring?

Reference

1. C. S. Lewis, *Mere Christianity* (Richard Bles, London, 1952).

10.1126/science.1133985

MOLECULAR BIOLOGY

From Magnetic Mines to DNA and Consciousness

Leslie Orgel

Francis Crick
Discoverer of the Genetic Code

by Matt Ridley

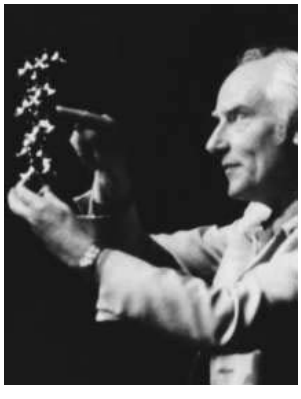
Atlas Books (HarperCollins), New York, 2006. 223 pp. $19.95, C$25.95. ISBN 0-06-082333-X. Eminent Lives.

Francis Crick, who died on 28 July 2004 at the age of 88, will be remembered by the general public for discovering, in collaboration with James Watson, the structure of DNA. Those with an interest in the history of science will also remember him for his role in the elucidation of the mechanism of protein synthesis and for his collaboration with Christof Koch that removed the taboo on the discussion of consciousness in the technical neuroscience literature. His friends from the Cambridge of the 1950s and 1960s, whether scientists or not, will remember vividly the Bohemian parties that he and his wife Odile hosted in their home at 19 Portugal Place. Science writer Matt Ridley has had to cover all of this and more, without the help of diagrams or photographs, within the constraints imposed by the format of the Eminent Lives series of short, nonspecialized biographies.

Crick had shown no signs of unusual intellectual excellence as a student before the outbreak of the Second World War. Ridley's account of his work on the design of magnetic mines seems to me to be particularly revealing. The idea of designing a mine that would sink minesweepers but not merchant ships was unconventional thinking at the time, although the story current in Cambridge that senior navy officers regarded it as unsporting must be apocryphal. Francis and his colleagues went on to calculate the sensitivity and other parameters that would be needed by analyzing photographic records of a German minesweeper exploding a mine in waters of known depth. He left it to others to engineer the mines.

There is little to add to what has already been written by Jim Watson and others on the events leading up to the discovery of the structure of DNA. Ridley provides a concise account of the factors that made the discovery possible. So many things could have changed the outcome. Without financial help from well-off Uncle Arthur, Francis might have had to give up science. If Rudolf Signer had not given a very pure DNA sample to Maurice Wilkins, Rosalind Franklin could not have obtained the critical x-ray photographs. If the atmosphere at Kings College had been more collegial, the structure might have been solved there, or Linus Pauling might have finished first if he had not been denied a visa to visit England. Without the help of Jerry Donohue, Watson might have gone on using the wrong tautomeric structures for the bases and failed to find the base pairs. Nonetheless, Ridley's account shows that Watson and Crick were much more than lucky. Unlike their competitors, they realized that the DNA structure was likely to be a key to understanding the nature of life and they wanted passionately to be the first to find it.

Ridley rightly emphasizes the key role that Crick played in working out the genetic code. Francis's own experiments established that a sequence of three nucleotides coded for a single amino acid. The adaptor hypothesis and wobble pairing were also important, but his relentless analysis of the accumulating experimental data, some of it misleading, was Francis's main contribution. He was certainly the conductor of the orchestra, but I think he would have given a great deal of credit to Marshall Nirenberg, Gobind Khorana, and the other soloists.



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The deciphering of the genetic code was Francis's last major contribution to molecular biology, and Ridley makes clear that his efforts for the next few years were not particularly productive scientifically. Brief forays into public affairs were on the whole a failure. He later admitted that he had been insensitive in his attempt to treat racial differences in intelligence as just another scientific problem. Francis needed a more fundamental scientific subject to work on. He found it in the problem of consciousness.

After moving permanently to the Salk Institute in 1977, Crick immersed himself in the literature of the neurosciences. His major achievement in his long collaboration with Koch was to make consciousness (or, more cautiously, the neural correlate of consciousness) a respectable topic for discussion in the scientific literature. It is not yet clear whether the framework that he and Cristof developed in their discussions of consciousness will be validated in the future.

Ridley has written a very readable book. He describes Crick's scientific achievements with remarkable clarity and gives a lively account of Francis and Odile's social life. I think Francis would have agreed that this little book provides an excellent framework for the more detailed biographies that are sure to follow.

10.1126/science.1132541

GENOMICS

An Ointment for the Fly

J. Craig Venter

Won for All: How the Drosophila *Genome Was Sequenced* is an odd, short, gossipy, emotional, abstract rendition of the Celera jamboree that gives much of the party line of good (government) versus evil (Celera Genomics). Cambridge University geneticist Michael Ashburner, like others, could not then and apparently still cannot accept the fact that our plan from the start was to publish both the fly and human genomes. Although Ashburner is never one to let facts get in the way of a good story and it is hard to agree with him on many of the views he expresses, I do applaud his tribute to the event and the wonderful team of scientists who made it all happen.

Won for All
How the *Drosophila* Genome Was Sequenced
by Michael Ashburner
Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2006. 123 pp. $19.95, £11. ISBN 0-87969-802-0.

The *Drosophila* genome annotation jamboree was held in November 1999 in Rockville, Maryland, at the conclusion of the highly successful four-month collaborative genome sequencing effort by Celera and the Berkeley *Drosophila* group. The gathering turned out to be a unique social experiment in genomics. Top *Drosophila* and bioinformatic scientists from around the world were invited to Celera to help annotate the just-sequenced and -assembled *Drosophila* genome (*1*). The idea was conceived of by Gerry Rubin, Mark Adams, and me as a means of providing a high-quality fly genome to the community in the shortest time possible.

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The collaborative effort to sequence the *Drosophila* genome had its inception only months before at Cold Spring Harbor, when I told Gerry Rubin that I wanted to sequence the fly genome for its biological importance and as a test project for the human genome. I asked if he would like to collaborate to get it done quickly. He accepted immediately, and the best scientific collaboration of my career was under way. Rubin's team had already completed 20 to 30 percent of the fly genome, which we thought would provide a great quality check against the independently produced shotgun assembly.

Because the genome community had been highly polarized by my announcement of Celera's formation to sequence the human genome, we wanted to reach out broadly in the community to be as inclusive as possible and to help change the views of those who objected to our efforts. We were specifically interested in involving British genome scientists, who seemed more incensed than most that a research team with commercial backing was entering their sacred world. Most of the scientists we recruited were easy choices (based on their accomplishments and areas of expertise), but a few caused me some concern. Ashburner seemed, in my view, an emotional and political liability, but Gerry insisted he was a must have if I was going to win over critics.

When Michael arrived, I met the excitable, hairy, chain-smoking gnome who behind the scenes had already created much turmoil over Gerry's decision to collaborate with me. Despite the prior furor, the author was a likable person who showed up on my doorstep willing to try my experiment. We made a bet at the start of the jamboree that if in the end he thought it was successful, he would pose for a photo on his hands and knees with my foot on his back. As they say, a picture tells a thousand words. We did what we said we would. The entire DNA sequence of *Drosophila* was deposited in GenBank a few months later, and our paper appeared less than a year from my asking Gerry to collaborate.

Rather than a detailed history of the private-public partnership to sequence the *Drosophila* genome, the book is more of an extended essay or diary, which runs for 75 small-format pages with two short, noncongruent assays (an epilogue by R. Scott Hawley and an afterword by Ethan Bier) added at the end. It is what anyone who knows Ashburner might expect: some history, some science, and a good deal of fun wrapped up in a continuous cloud of smoke. If you want a quick read that adds some color to other accounts of the efforts to sequence the fly and human genomes, then it is worth checking out *Won for All*.

Result of the bet.

CREDIT: MARTY KATZ

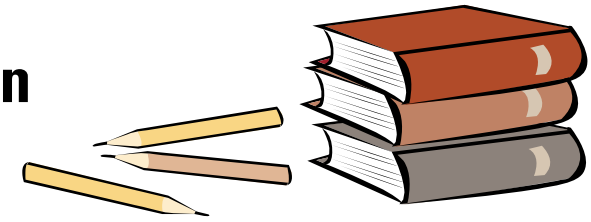
Reference

1. M. D. Adams *et al.*, *Science* **287**, 2185 (2000).

10.1126/science.1134998

THE EARLY YEARS

Evaluating Montessori Education



Angeline Lillard[1]* and Nicole Else-Quest[2]

An analysis of students' academic and social scores compares a Montessori school with other elementary school education programs.

Montessori education is a 100-year-old method of schooling that was first used with impoverished preschool children in Rome. The program continues to grow in popularity. Estimates indicate that more than 5000 schools in the United States—including 300 public schools and some high schools—use the Montessori program. Montessori education is characterized by multi-age classrooms, a special set of educational materials, student-chosen work in long time blocks, collaboration, the absence of grades and tests, and individual and small group instruction in both academic and social skills (*1*). The effectiveness of some of these elements is supported by research on human learning (*2*).

We evaluated the social and academic impact of Montessori education. Children were studied near the end of the two most widely implemented levels of Montessori education: primary (3- to 6-year-olds) and elementary (6- to 12-year-olds). The Montessori school we studied [located in Milwaukee, Wisconsin (*3*)], which served mainly urban minority children, was in its ninth year of operation and was recognized by the U.S. branch of the Association Montessori Internationale (AMI/USA) for its good implementation of Montessori principles (*4*).

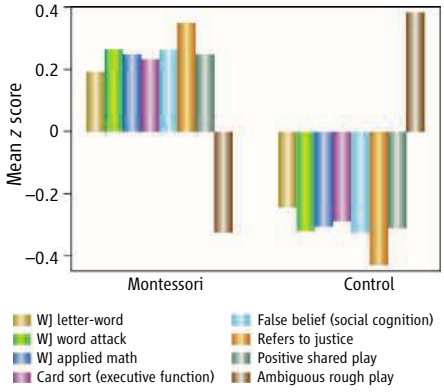
Because it was not feasible to randomly assign children to experimental and control educational groups, we designed our study around the school lottery already in place. Both the experimental and the control group had entered the Montessori school lottery; those who were accepted were assigned to the experimental (Montessori) group, and those who were not accepted were assigned to the control (other education systems) group. This strategy addressed the concern that parents who seek to enroll their child in a Montessori school are different from parents who do not. It is crucial to control for this potential source of bias, because parents are the dominant influence on child outcomes (*5*).

Recruitment

We contacted parents of children who had entered the Montessori school lottery in 1997 and 2003 and invited them to be in the study. All families were offered $100 for participation.

Because the lottery, which was conducted by the school district, was random, the Montessori and control groups should contain similar children. Ninety percent of consenting parents filled out a demographic survey. Parents from the Montessori and control groups had similar average incomes ($20,000 to $50,000 per year) at each student age level. This addressed a concern with a retrospective lottery loser design that the final samples might be different for reasons other than the treatment. Another variable, ethnicity, was not surveyed because parent income contributes more to child outcomes than does ethnicity (*6*). We were also concerned that requesting ethnicity data would reduce participation in this racially divided city.

Overall, 53 control and 59 Montessori students were studied (table S1). The 5-year-old group included 25 control and 30 Montessori children, and the 12-year-old group included 28 control and 29 Montessori children. Gender balance was imperfect, but gender did not contribute significantly to any of the differences reported here. Children at the Montessori school were drawn from all six classrooms at the primary level and all four at the upper elementary level. The control children were at non-Montessori schools: 27 public inner city schools (40 children) and 12 suburban public, private/voucher, or charter schools (13 children). Many of the public schools had enacted special programs, such as gifted and talented curricula, language immersion, arts, and discovery learning.



Results for 5-year-olds. Montessori students achieved higher scores [converted to average *z* scores (*18*)] for both academic and behavioral tests.

Children in both groups were tested for cognitive/academic and social/behavioral skills that were selected for importance in life, not to examine specific expected effects of Montessori education. Our results revealed significant advantages for the Montessori group over the control group for both age groups.

Results: 5-Year-Olds

Cognitive/Academic Measures. Seven scales were administered from the Woodcock-Johnson (WJ III) Test Battery (*7*). Significant differences favoring Montessori 5-year-olds were found on three WJ tests measuring academic skills related to school readiness: Letter-Word Identification, Word Attack (phonological decoding ability), and Applied Problems (math skills) (see chart, left). No difference was expected or found on the Picture Vocabulary test (basic vocabulary) because vocabulary is highly related to family background variables (*8*). Two WJ tests of basic thinking skills—Spatial Reasoning and Concept Formation—also showed no difference.

Five-year-olds were also tested on executive function, thought to be important to success in school. On one such test, children were asked to sort cards by one rule, switch to a new rule, and (if they did well) then switch to a compound rule. Montessori children performed significantly better on this test. A test of children's ability to delay gratification (a treat) did not indicate statistically significant differences.

Social/Behavioral Measures. Children were given five stories about social problems, such as another child hoarding a swing, and were asked how they would solve each problem (*9*).

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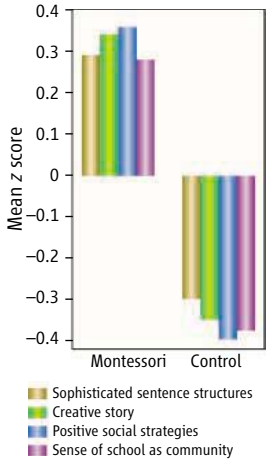
Montessori children were significantly more likely (43% versus 18% of responses) to use a higher level of reasoning by referring to justice or fairness to convince the other child to relinquish the object. Observations at the playground during recess indicated Montessori children were significantly more likely to be involved in positive shared peer play and significantly less likely to be involved in rough play that was ambiguous in intent (such as wrestling without smiling).

The False Belief task was administered to examine children's understanding of the mind (*10*). Recognition that people represent the world in subjective as well as objective ways is a landmark achievement in social cognition (*11*). Social negotiation and discussion about mental states leads to this advance in children (*12*). Whereas 80% (significantly more than chance) of the Montessori 5-year-olds passed, the control children were at chance, with 50% passing.

Results: 12-Year-Olds

Cognitive/Academic Measures. Twelve-year-olds were given 5 minutes to complete a story beginning "____ had the best/worst day at school." The Montessori students' essays were rated as significantly more creative and as using significantly more sophisticated sentence structures (see chart, below). Control and Montessori essays were similar in spelling, punctuation, and grammar. Unlike the 5-year-olds, the 12-year-olds did not perform differently on the WJ tests. This is surprising, because early reading skills normally predict later reading (*13*). Either the control group had "caught up" by age 12 to the Montessori children, or the 12-year-old Montessori children were not more advanced in these early reading skills when they were 5. If the latter, one possible explanation is that the 12-year-olds started at the school when it was in its third year. The Montessori method relies on peer teaching and modeling, so those who are in the early classes of a new school lack some advantages relative to those who begin later.

Social/Behavioral Measures. As a social skills test, 12-year-olds read six stories about social problems (such as not being asked to a party) and were asked to choose among four responses. Montessori 12-year-olds were significantly more likely to choose the positive assertive response (for example, verbally expressing one's hurt feelings to the host). On a questionnaire regarding their feelings about school, Montessori children indicated having a greater sense of community, responding more positively to items such as, "Students in my class really care about each other" and "Students in this class treat each other with respect."



Results for 12-year-olds. Students in the Montessori program wrote more sophisticated and creative stories and showed a more developed sense of community and social skills. Scores were converted to average *z* scores (*18*).

Benefits of Montessori Education

On several dimensions, children at a public inner city Montessori school had superior outcomes relative to a sample of Montessori applicants who, because of a random lottery, attended other schools. By the end of kindergarten, the Montessori children performed better on standardized tests of reading and math, engaged in more positive interaction on the playground, and showed more advanced social cognition and executive control. They also showed more concern for fairness and justice. At the end of elementary school, Montessori children wrote more creative essays with more complex sentence structures, selected more positive responses to social dilemmas, and reported feeling more of a sense of community at their school.

These findings were obtained with a lottery loser design that provides control for parental influence. Normally parental influence (both genetic and environmental) dominates over influences such as current or past school and day-care environments. For example, in the large National Institute of Child Health and Human Development (NICHD) study of early child care, correlations between parenting quality and WJ early academic tests had effect sizes comparable to those seen here, whereas school effects were much smaller (*5*). An evaluation of *Success for All*, considered a highly successful reading intervention, reported a quarter of a standard deviation as its largest effect size (for Word Attack) in a randomized field trial, and stated that it was equal to a 4.69-month advance in reading skills (*14*). Stronger effects are often found in the first years of pilot programs when researchers are involved in implementation of their own programs (*15*), termed the "super-realization effect" (*16*). In our study, the school did not anticipate an evaluation. Especially remarkable outcomes of the Montessori education are the social effects, which are generally dominated by the home environment (*17*).

Future research could improve on the research design here by following lottery participants prospectively and by tracking those who drop out and examining their reasons. It would be useful to replicate these findings in different Montessori schools, which can vary widely. The school involved here was affiliated with AMI/USA, which has a traditional and relatively strict implementation. It would also be useful to know whether certain components of Montessori (e.g., the materials or the opportunities for collaborative work) are associated with particular outcomes.

Montessori education has a fundamentally different structure from traditional education. At least when strictly implemented, Montessori education fosters social and academic skills that are equal or superior to those fostered by a pool of other types of schools.

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19. Funding was provided by the Jacobs and Cantus Foundations and sabbatical fellowships from the Cattell Foundation and the University of Virginia to A.L. J. DeLoache, B. Detmer, L. Ma, A. Pinkham, R. Tai, and J. van Reet provided helpful comments, and E. Turkheimer provided valuable statistical advice. We thank the Milwaukee schools that participated; the children and their families; and A. Hart, T. Nishida, A. Pinkham, J. van Reet, and B. Rosen.

Supporting Online Material

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APPLIED PHYSICS

Tuning Interface States

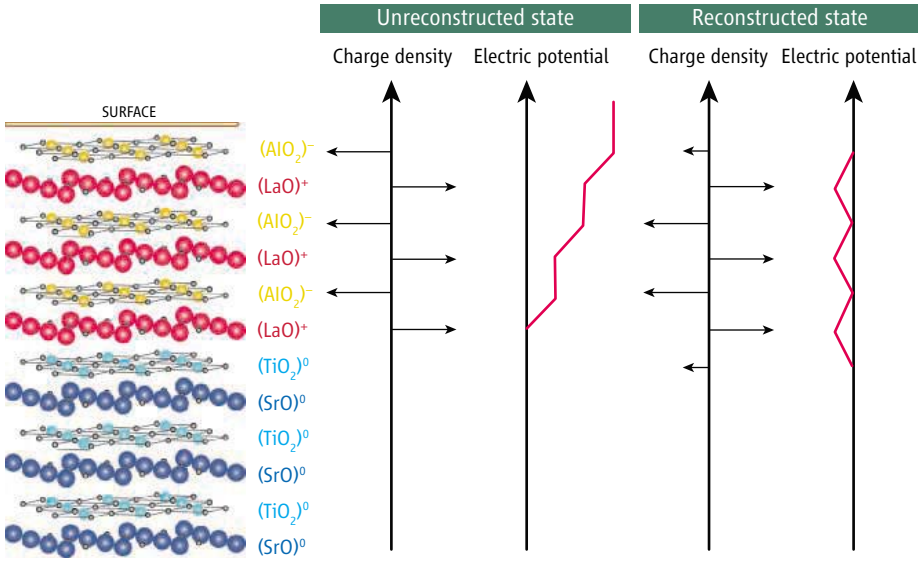
Harold Y. Hwang

When grown with atomic precision, materials that are normally insulators can form a conducting interface with properties that can be controlled by an external field.

An important goal of nanoscience research is the creation of new functionality by controlling materials down to the atomic level. An emerging example can be found in recent studies of the electronic structure at the interface between perovskite oxides. Modern atomic-scale growth and probe techniques enable the formation and study of new artificial interface states that are quite distinct from the bulk state. For example, the interface between two insulators, $LaAlO_3$ and $SrTiO_3$, sustains a metallic phase with high carrier mobility for an appropriate atomic arrangement of the interface (*1*). This is analogous to modulation doping in GaAs heterostructures and provides a new avenue for doping perovskite interfaces, accessing the diverse physical properties of these materials, including superconductivity, magnetism, ferroelectricity, and their intercouplings. On page 1942 of this issue, Thiel *et al.* (*2*) report that this interface can be dynamically tuned across a metal-insulator transition by applying an external gate field. The result is a system that can be switched from highly insulating to highly conducting for a wide range of potential device applications.

The interface in question represents the border between stacks of charge-neutral atomic layers in $SrTiO_3$, and alternately charged atomic layers in $LaAlO_3$ (see the figure). The electrostatic potential produced by the charges diverges with $LaAlO_3$ thickness (assuming that there are no charge rearrangements compared to the bulk structure). This divergence represents a truly energetically unfavorable circumstance—it quickly becomes the dominant energy in the system, and some reconstruction must occur.

Such a polar discontinuity is not at all new; it has been much discussed for semiconductor heterointerfaces (*3*–*5*). In those cases, the interface atomically reconstructs, changing the interface stoichiometry (usually accompanied by considerable roughening and diffusion) to remove the diverging potential. What is new here is the realization that the potential divergence can be overcome by an electronic reconstruction. Unlike semiconductors, transition metal ions at the interface can acquire a mixed-valence ionic character, whose charge balances the polar discontinuity. In the specific case shown in the figure, the titanium ions, normally Ti^{4+} elsewhere, tend toward Ti^{3+} at the interface, inducing a quasi–two dimensional electron gas (*6*). It should also be noted that the polar surface must also reconstruct, although the detailed configuration is not currently known.



Polar discontinuities. **(Left)** The unreconstructed interface examined by Thiel *et al.* **(Middle)** This charge configuration leads to an electric potential that diverges with $LaAlO_3$ thickness. **(Right)** Above a threshold thickness, the charge distribution reconstructs, removing the divergence. This is shown as a layer of $Ti^{3.5+}$ at the interface, and a simple reconstruction of the polar surface of $LaAlO_3$. More realistic charge distributions can be described by incorporating additional dipoles, but the overall structure remains unchanged.

Thiel *et al.* initially observe that there is a threshold thickness for the electronic reconstruction to occur. For $LaAlO_3$ layers that are up to three unit cells thick, the interface is insulating. When one or more unit cells of $LaAlO_3$ are added, the interface becomes abruptly metallic. Their results imply a crossover between the unreconstructed and reconstructed state as a function of interface thickness (*7*). This is similar to recent observations of proximity coupling of polar discontinuities with opposite sign (*8*)—that is, coupling between two interfaces, rather than between an interface and a surface. Next, Thiel *et al.* find that this balance point can be shifted by applying an external gate voltage, inducing a metal-insulator transition observed both at room temperature and at 4.2 K. This shift is quite dramatic, with changes in interface conductance exceeding seven orders of magnitude (limited by detection of the insulating state).

Taken together, these results demonstrate substantial progress in the manipulation of new artificial low-dimensional charge states formed at oxide heterointerfaces. What is exciting is that extremely high doping levels can be achieved by polar discontinuities, notably exceeding the largest values obtained for field effect transistors, ferroelectric gate devices, and photocarrier generation. The figure implies an interface carrier density of ~6×10^{14} cm^{-2}; although such values have been observed, the carrier density can be varied very widely depending on growth conditions (the structures of Thiel *et al.* show an interface carrier density of ~2×10^{13} cm^{-2}, and were optimized for lower carrier density). Growth kinetics and defect chemistry are important for any experimentally realized interface, in which local rearrangements and vacancies can partially compensate the anticipated electronic structure. Indeed, it has been reported that in different growth conditions, the interface carrier density can be dominated by growth-induced oxygen vacancies (*9*, *10*).

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By growing precise structures incorporating an external gate, the interface can be statically and dynamically tuned over many orders of magnitude. At the high end, the doping levels should allow access to phase transitions that have not been previously reachable. These phase transitions can be precisely traversed and studied, as demonstrated by Thiel *et al.* Control of the interface electronic structure is central to the function and improvement of virtually all existing oxide device characteristics—similar considerations have already played a role in enhancing magnetic tunnel junctions, as an example (*11*). These and other advances are rapidly opening a new frontier in the science and technology of oxide heterostructures.

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GEOCHEMISTRY

Manganese Redox Chemistry Revisited

Manganese in natural oxygen-poor waters can persist in a +3 oxidation state, a state previously seen only in the lab, necessitating a major revision of the current understanding of manganese aqueous geochemistry.

Kenneth S. Johnson

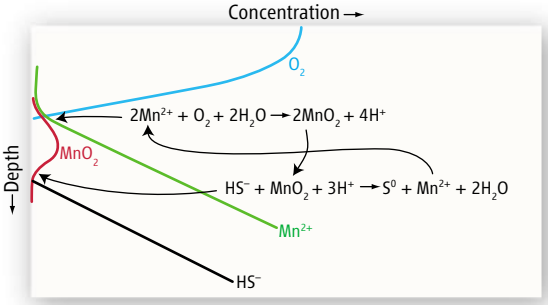
Manganese is an important component of some marine reduction-oxidation cycles. It is usually assumed that only the Mn(II) and Mn(IV) oxidation states play an important role in these cycles. But recently, Webb *et al.* (*1*) suggested that during the oxidation of Mn(II) by bacteria, Mn(III) is formed as an intermediate. Large concentrations of Mn(III) would then accumulate and leak into the environment. On page 1955 of this issue, Trouwborst *et al.* (*2*) show that in regions of the Black Sea and Chesapeake Bay where the concentration of molecular oxygen is extremely low (suboxic regions), much of the dissolved manganese is indeed present as Mn(III). This discovery will alter the paradigm on which our understanding of manganese aqueous geochemistry is based.

Manganese acts as a catalyst that shapes chemical gradients in the oxygen-deficient zones found throughout the coastal ocean and marginal seas. It can perform this role because it exists in multiple oxidation states and is recycled rapidly between these states by bacterial processes. These transformations serve as an electron-transfer system for other chemical cycles. For example, oxidized manganese consumes upwelling hydrogen sulfide in the Black Sea, which contains the world's largest mass of anoxic water (*3*). The reduced manganese produced during oxidation then diffuses upward, where it consumes downwelling oxygen (see the figure). This reaction resupplies the pool of oxidized manganese, thereby creating a catalytic cycle and a suboxic zone in which molecular oxygen and sulfide concentrations are very low.

Our understanding of how manganese mediates chemistry in these suboxic areas has been based on the paradigm that there are two predominant forms of manganese in the environment: dissolved, reduced Mn(II) and particulate, oxidized MnO_x [where x is near 2 and most of the manganese is in the +4 oxidation state, although particulate Mn(III) also exists]. The vertical or lateral gradients in dissolved or particulate manganese set limits on the rates of reaction that can occur. These gradients, multiplied by the appropriate diffusion coefficient or, in the case of particulate MnO_x, the appropriate settling velocity, define the flux of electron donors and acceptors available for reaction.

Dissolved Mn(III) has been largely ignored, because it is both a very strong oxidant and reductant that is expected to rapidly disproportionate to Mn(II) and MnO_2. The Mn(III) detected by Trouwborst *et al.* persists because it is stabilized by dissolved ligands, perhaps pyrophosphate. The authors measured the Mn(III) concentration by using desferrioxamine-B (DEF-B), an Fe^{3+}-binding ligand that is produced by bacteria. DEF-B also strongly binds Mn(III) and can outcompete natural ligands to sequester all of the dissolved Mn(III) in a filtered sample. The concentration of the DEF-B–Mn(III) complex can then be determined electrochemically.

In the Black Sea, water below a depth of about 100 m is permanently anoxic. The Chesapeake Bay is much shallower (~20 m), and anoxic zones form below ~15 m in the summer in response to nutrient-stimulated inputs of decaying phytoplankton. These two environments represent the extremes in anoxic conditions found in natural waters, one permanent and the other temporary. Dissolved Mn(III) was observed in both loca-



How manganese catalyzes chemical cycles in the Black Sea. In subsurface waters, Mn(II) reacts with oxygen, depleting its concentration and forming manganese oxide (MnO_2) particles. The particles sink into deeper waters, where they react with upwelling hydrogen sulfide, resulting in the regeneration of Mn(II). Trouwborst *et al.* report that much of the dissolved manganese in the suboxic zone is Mn(III), rather than Mn(II). This finding will require a reevaluation of how such cycles operate.

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tions in the suboxic zones, where both molecular oxygen and sulfide are nearly absent. In some cases, all of the dissolved manganese in the suboxic zone was in the +3 oxidation state, reaching concentrations of up to 4 μM.

Thermodynamic considerations indicate that manganese may play a dominant role as a catalyst in chemical cycles other than the HS^-/O_2 cycle shown in the figure. For example, the reduction of nitrate by Mn(II) and the oxidation of ammonia by MnO_2 are thermodynamically favorable in suboxic marine environments (*4*). Oxygen and Mn(II) concentrations do not seem to overlap in many sediment profiles, providing indirect evidence for the oxidation of Mn(II) in the absence of O_2. However, these reactions have been difficult to demonstrate with direct measurements in environmental samples (*5*).

The analytical tools used in these studies are based on the assumption that the dissolved manganese is all in the reduced form. If Mn(II) is oxidized in a one-electron step to Mn(III), then the concentration of dissolved Mn [which is the sum of Mn(II) and Mn(III) in most analytical methods] will not change. The classical paradigm would indicate that no reaction had taken place. This situation resembles our understanding of anaerobic methane oxidation a decade ago. At that time, field data suggested that a reaction consuming methane occurred, but the mechanism became clear only when new methodologies were developed (*6*). The work of Trouwborst *et al.* is a crucial step in moving us beyond the stalemate in our understanding of manganese cycling.

The discovery of substantial amounts of Mn(III) may also have implications for regions of the ocean that are not suboxic. The concentration of dissolved manganese decays smoothly in the deep (~3000 m) waters that flow along the oceanic conveyor from the North Atlantic into the Pacific. After several hundred radiocarbon years, manganese reaches a uniform concentration of 0.15 nM in these waters (*7*). In the case of Fe^{3+}, ligands such as DEF-B ("siderophores") act as a control that drives iron concentrations in the deep ocean toward uniform values (*8*). There has been little plausible evidence for similar processes involving dissolved manganese, because Mn(II) interacts with organic ligands much more weakly than does Fe^{3+}. The evidence for substantial production of Mn(III) and its capability to interact strongly with siderophores introduces a new mechanism for sustaining uniform manganese concentrations in the deep ocean.

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GENOMICS

Genomics and the Tree of Life

The wealth of genome sequences—from more than 400 organisms to date—has enriched the evolutionary tree, but has also presented new challenges.

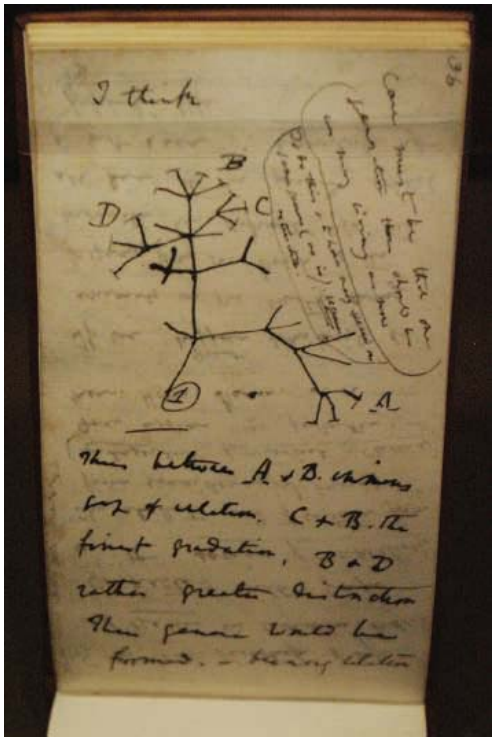
Antonis Rokas

Only a decade has elapsed since the first prokaryote and eukaryote genomes were decoded. More than 400 genomes have been completed, some 1600 additional genomes are currently in progress, and genome-scale data sets (e.g., expressed sequence tags) are being generated at an unprecedented rate. Among the many fields feeling the impact of this genomic avalanche is phylogenetics, the discipline concerned with discovering the evolutionary interrelationships among all living organisms, an effort frequently visualized in the form of the Tree of Life (see the figure) (*1*). The wealth of genomic data has allowed the discovery of new molecular markers for phylogenetic reconstruction, such as rare genomic changes, but it has also presented new challenges for theoretical phylogenetic research.

The dramatic increase in data set sizes has led, in many cases, to increased confidence in the inference of evolutionary relationships (*2*, *3*). Data sets with small gene numbers can generate inaccurate phylogenies because of sampling error or simply the lack of sufficient amounts of data (*3*). Although typical genome-scale phylogenetic studies have been rich in sequence data and thin in species number (*3*), as the number of sequenced genomes increases, genome-scale phylogenetic analyses are beginning to feature much larger numbers of species (*4*).

But further increases in data set sizes present challenges as well. Analyzing many thousands of nucleotides for hundreds or thousands of species requires substantial computational power to efficiently search among all possible trees (*5*). More sophisticated statistical algorithms are also needed for discovering the trees best supported by the data (*6*). Several clades of the Tree of Life, including the one of Metazoa, are proving difficult to resolve too (*7*). Most parameters of sequence evolution vary across lineages. Slight biases—amplified by the sheer volume of data—can potentially mislead phylogenetic algorithms and provide high support for the wrong trees.

Whereas the linear information in genome sequences may not always suffice, other rare features in the genomes' contents, such as sequence rearrangements or integrations of



A lovely tree. Charles Darwin's famous notebook B containing the first known sketch of an evolutionary tree.

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mobile genetic elements, offer some powerful alternative markers for addressing such challenging phylogenetic riddles. The use of such rare genomic changes is feasible only in a genomic context and can frequently yield remarkably precise evolutionary trees. In the mammalian lineage, this approach has led to the discovery of several new clades such as the Pegasoferae, which unexpectedly combines bats with horses, cats, dogs, and pangolins (*8*). Even though the use of rare genomic changes is still in its infancy, the first steps toward placing rare genomic changes–based phylogenetic reconstruction in a robust statistical framework have already been taken, thus allowing a better evaluation of their usefulness in phylogenetic reconstruction (*9*).

Genomics has also brought into sharp focus some thorny topics, such as the evolutionary impact of lineage sorting of ancestral genetic polymorphisms (*10*) and lateral gene transfer (*11*) on phylogenetic reconstruction. Although lineage sorting can be addressed by careful phylogenetic study design, the extensive occurrence of lateral gene transfer in prokaryotes has raised concerns as to the validity of any gene-based phylogenies for these organisms (*11*). Instead, gene histories in genomes that have undergone lateral gene transfer are more likely to resemble evolutionary networks and not trees. In fact, consideration of the effect of lateral gene transfer has led to searches for core sets of genes that share the same evolutionary history and are thus likely recalcitrant to transfer between organisms (*12*). Such knowledge of a core set of orthologs is crucial for phylogenetic purposes, as it allows a precise estimate of the maximum amount of data available for use in evolutionary analyses. For example, it has been estimated that only 80 out of thousands of genes can be identified as orthologs across all Bacteria, Archaea, and Eucarya (the three domains of the Tree of Life) (*12*), a value not far from the gene number some current studies are using (*4*). However, given the prevalence of lateral gene transfer—mostly in prokaryotes, to a much lesser extent in eukaryotes—as well as the high frequency of gene gain and loss, questions as to whether these core gene sets are meaningful or how we can reliably identify orthology among genes remain wide open (*11*).

The integration of genomics data into the phylogenetics mold is just beginning. As the choice of genomes to be sequenced is increasingly guided by evolutionary considerations, and as emerging sequencing technologies promise to drop costs even lower, the reach and impact of genomics to non–model organisms is rapidly extending. Of course, with about 2 million known species of organisms and another 10,000 being discovered each year, the fraction of species for which genome-scale data are available is truly minuscule. Although phylogeneticists have been publishing an average of 15 phylogenetic trees per day, less than 1% of known species have been part of any sort of phylogenetic analysis (*1*). Given the breadth of organismal diversity, the gene-scale era of phylogenetics is still an invaluable asset to the pursuit of the Tree if Life. Comparative genomics, with its ability and potential to vastly increase both the amount and type of molecular data available for a small but critical fraction of biodiversity, is bound to play an increasingly important role in efforts to assemble a robust picture of the Tree of Life.

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PLANETARY SCIENCE

Merging Views on Mars

Rover observations and global satellite data show that the surface of Mars was wet and acidic early in its history, but rapidly became dry and oxidizing.

Jean-Pierre Bibring, Steven W. Squyres, Raymond E. Arvidson

In 2003, three spacecraft went to Mars. One was the European Space Agency's Mars Express orbiter, whose primary goal was to map the planet with a suite of remote sensing instruments. The other two were Spirit and Opportunity, the two rovers of NASA's Mars Exploration Rover (MER) mission. Their job was to explore two locations on the martian surface in detail, searching for evidence of past environmental conditions and their suitability for life.

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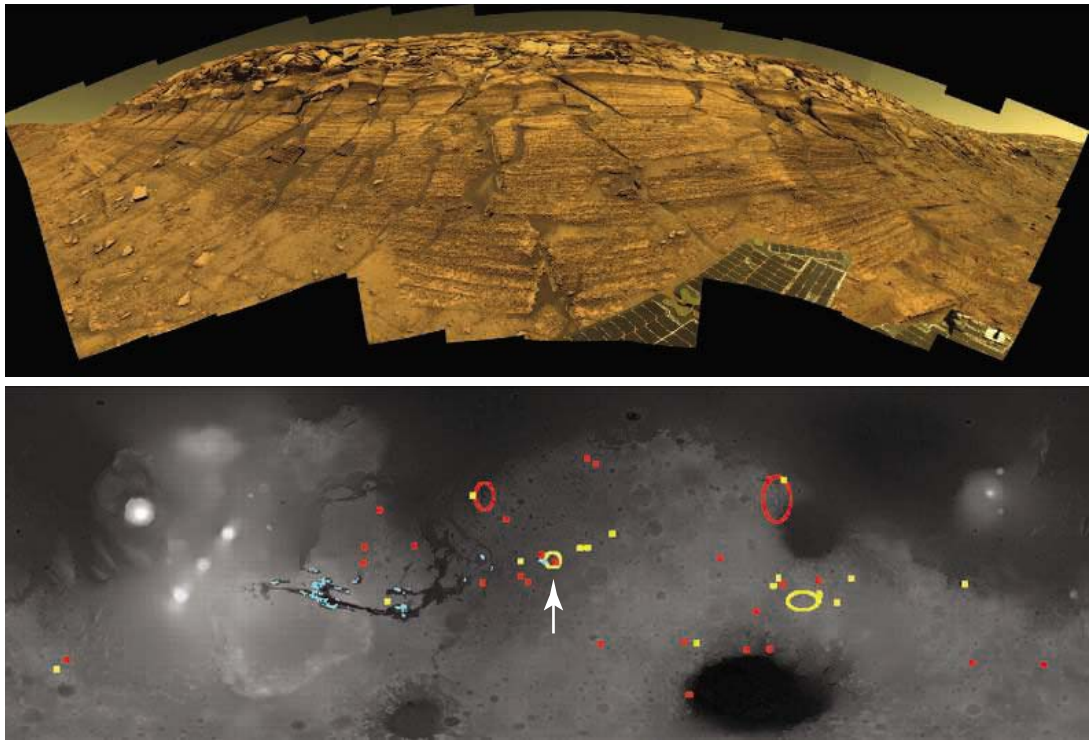
Three years after launch, and more than 2 years after arrival at Mars, all three spacecraft are still going strong. Along the way, they have acquired complementary data sets that reveal new information about the history of martian water and the possibility that the planet once harbored habitable conditions. Recently scientists from the OMEGA (Observatoire pour la Mineralogie, l'Eau, les Glaces et l'Activité) spectral imager team and the MER Athena science team met together to compare and merge results from their two investigations for the first time (*1*). Each group brought data from a sophisticated array of instruments and detectors (*2*, *3*) to the workshop.

Spirit landed in Gusev crater on 4 January 2004. Gusev was chosen because a large dendritic channel, Ma'adim Vallis, debouches into it, suggesting that a lake once occupied the crater. The rover, however, did not find sedimentary rocks at its plains landing site. Instead, all of the rocks there were olivine-rich basalts, indicating that any lacustrine sediments had subsequently been buried with lava. The basalts were largely unaltered, implying little aqueous activity at the site since their emplacement. Spirit spent all of its 90-sol (*4*) originally scheduled mission exploring lava plains (*5*).

Circumstances changed on sol 156, when Spirit reached the base of the Columbia Hills, 2.5 km from the landing site. These hills, which predate the lava plains, show remarkable geologic diversity. In more than 700 sols of exploration, Spirit has identified more than 10 chemically distinct rock types there. Most are impact ejecta or volcanic materials, many

of which have undergone substantial alteration. Concentrations of sulfur, chlorine, phosphorus, and bromine are high in some rocks, and the iron oxyhydroxide mineral goethite implicates water in the alteration process. One rare rock type is a basaltic sandstone cemented by 15 to 20% magnesium sulfates and calcium sulfates, perhaps pointing to a localized episode of surface water. Spirit's results reveal a brief period early in martian history when impacts were frequent and subsurface water was present, followed by emplacement of plains lavas. After lava emplacement, Gusev has been geologically quiescent, under cold, dry environmental conditions.



Complementary maps. (**Top**) A panoramic image acquired by the Opportunity rover of Burns Cliff, a prominent outcrop forming the rim of Endurance crater. The image shows a sequence several meters thick of finely layered sulfate-rich sediments that contain small hematite-rich concretions. (**Bottom**) A global topographic map of Mars, with mineral occurrences detected from orbit by OMEGA. Blue denotes sulfates, red denotes phyllosilicates, and yellow denotes other hydrated phases. Arrow indicates the location of the Opportunity landing site.

The second MER rover, Opportunity, landed at Meridiani Planum on 25 January 2004. Meridiani was chosen because orbital observations by the Thermal Emission Spectrometer on the Mars Global Surveyor orbiter had detected coarse-grained hematite, a mineral that can form through aqueous processes.

The rover landed in Eagle crater, a small impact crater. Exposed in its walls are outcrops of finely layered sandstone. The sand grains composing the outcrops are nearly 40% by weight sulfate salts, including magnesium sulfate, calcium sulfate, and the iron sulfate jarosite, which normally forms at low pH. Embedded in the rocks are hematite-rich spherules 4 to 6 mm in diameter, interpreted to be concretions formed by precipitation from groundwater. A surface lag deposit of concretions produces the hematite signal seen from orbit. Layering in Eagle crater shows centimeter-scale concave-upward or "festoon" geometry cross-bedding, which is diagnostic of deposition in flowing surface water (*6*, *7*).

Leaving Eagle crater, Opportunity traversed to Endurance crater, a much larger crater ~800 m to the east (see the figure). Opportunity spent more than 10 months there, examining a ~7-m stratigraphic section. The whole section consists of concretion-laden, sulfate-rich sandstone. Most of it was deposited by wind, with evidence for deposition in surface water only in the upper ~0.5 m. Chemical and textural changes in the lower part of the section indicate diagenesis (that is, change after deposition) induced by interaction with groundwater. Opportunity's results indicate that acidic groundwater was once abundant at Meridiani, whereas conditions at the surface were oxidizing and generally arid. Sulfate-rich sands formed by interaction of acidic fluids with a precursor basaltic material were reworked by wind, and locally by surface water.

Mars Express arrived at Mars on 25 December 2003. Since then, the OMEGA imager has revealed substantial mineralogic diversity over the planet's surface (*8*, *9*). Iron-bearing pyroxene is common; both high-calcium pyroxene (HCP) and low-calcium pyroxene (LCP) have been detected and mapped. LCP-rich materials are common within heavily cratered primordial crust, whereas HCP-rich materials are prevalent in younger lava flows. Olivine is associated with both types of pyroxene, especially near impact craters. OMEGA data for the plains of Gusev show a dusty surface with underlying basaltic sand, consistent with Spirit observations.

The OMEGA team discovered phyllosilicates, primarily iron/magnesium smectites, in several locations (see the figure). All of these are restricted to ancient Noachian terrains, suggesting that phyllosilicate formation may have taken place primarily during the earliest portion of martian history. Phyllosilicates have also been inferred from rover observations in some rocks of the Columbia Hills on the basis of elemental chemistry, and the phyllosilicate nontronite has been suggested as a possible component of the Meridiani outcrop rocks on the basis of infrared spectrometer data.

Sulfates were also detected by OMEGA to the north of Opportunity's landing site, in a region where erosion has extensively exposed the types of rocks found in craters by the rover. This result indicates that sulfates are prominent in layered rocks that cover a considerably larger area than the hematite-rich lag deposit upon which Opportunity landed. Interestingly, sulfates are also common in layered deposits within the Valles Marineris, and ferric oxides have been detected there by OMEGA as well. These observations suggest that the aqueous processes responsible for sulfate and hematite formation at Meridiani may have operated elsewhere on Mars, including the Valles Marineris.

OMEGA has found evidence for widespread anhydrous ferric oxides on Mars, particularly in high-albedo regions. Candidate minerals include nanocrystalline red hematite

(α-FeO) and maghemite (γ-FeO). These results are in accord with MER Mössbauer results that show nanophase oxides in bright soils at both landing sites. The widespread nature of these materials and the lack of evidence for hydration suggest that they formed primarily under dry, oxidizing conditions that have prevailed for much of martian history.

The results from OMEGA and MER suggest that surface water may have led to production of phyllosilicates early in martian history, and perhaps somewhat later to deposition of hydrated sulfates. Because phyllosilicates and sulfates are found in different parts of the planet, the extent to which their formation might have overlapped in time is an important subject for future work. The acidity that promoted sulfate precipitation likely resulted from sulfur outgassing during volcanic activity. Both the roughly neutral pH suggested by phyllosilicates and the lower pH suggested by sulfates could have produced habitable surface environments; the former may have been more suitable for the origin of life. A future mission to phyllosilicate-rich terrains, followed by sample return from whichever terrain type shows the best overall potential for preservation of biosignatures, could be a good strategy for future Mars exploration. The combined results of the OMEGA and MER investigations illustrate how important international collaboration and associated synergistic analyses could be in such a venture.

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10.1126/science.1132311

CHEMISTRY

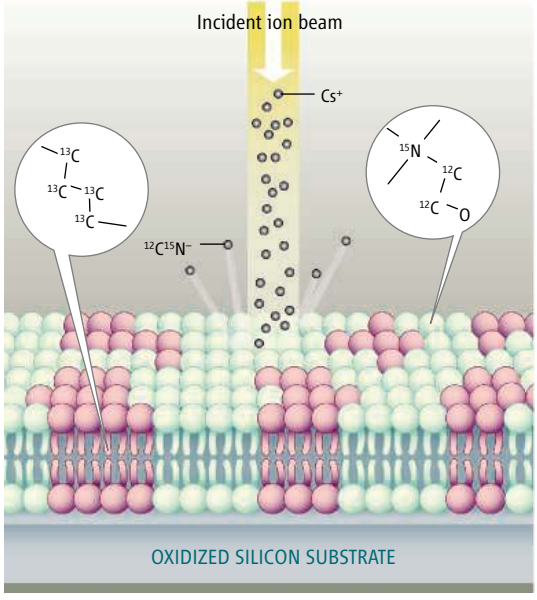
Unveiling the Membrane Domains

A new form of mass spectrometry can determine a membrane's chemical composition with a resolution of less than 100 nanometers.

Jay T. Groves

Cell membranes consist of a richly heterogeneous fluid mosaic of lipids and proteins. Molecular complexes, from tens to hundreds of nanometers in size, dynamically assemble and dissolve while performing the biochemical functions of life. But the spatial organization of the cell membrane and its role in the regulation of biochemical processes remain little understood, because current imaging technologies cannot resolve the most important features. On page 1948 of this issue, Kraft *et al.* take the next step in biomembrane imaging by using a form of secondary ion mass spectrometry (NanoSIMS) to map the chemical composition of a lipid membrane with 70 to 100 nm resolution (*1*).

Present knowledge of the organization of living cells comes mostly from fluorescence and electron microscopy. Over the past 20 years, fluorescence microscopy has flourished, due in part to the introduction of an extensive array of fluorescent probe molecules. By synthetically or genetically—such as with green fluorescent protein—coupling a fluorescent probe to the protein of interest, the ambiguity of what is being observed is broken. The ability to track a specific protein in living cells has revealed a tremendous wealth of information about the inner workings of biological systems. However, the spatial resolution of optical imaging techniques is generally restricted to a few hundred nanometers. Electron microscopy can be used to view cellular structures down to molecular length scales, allowing the bilayer structure of lipid membranes to be directly imaged. However, the lateral organization within the membrane has been more difficult to resolve. The need for imaging biomembrane organization at length scales of 10 to 300 nm thus remains largely unmet.



A detailed look at membrane composition. In the NanoSIMS experiments reported by Kraft *et al.*, a focused beam of ions bombards the sample, releasing a barrage of small ions that are analyzed with mass spectrometry. By scanning over the bilayer membrane sample, a high-resolution (about 70 to 100 nm) image of its chemical composition is generated.

Imaging mass spectrometry has the potential to step into this resolution gap. In recent years, this technique, which offers unparalleled chemical specificity, has been increasingly used to study biological systems (*2*, *3*). There are several ways in which mass spectrometry may be performed in a spatially resolved manner. In matrix-assisted laser desorption ionization (MALDI), a focused laser spot is scanned over a sample that has been prepared in a chemical matrix. The method produces relatively large molecular ions and enables direct identification of peptides and proteins without the need for specific labeling. Imaging MALDI has been successfully applied to biological tissue samples and has been used as a bioanalytical tool in array-based protein assays (*4*–*6*). However, the spatial resolution is typically limited by the laser spot size (about 1 μm).

Secondary ion mass spectrometry (SIMS) provides an alternative strategy. In this method, the sample is bombarded with an incident ion or molecular beam. The beam locally vaporizes the sample into secondary molecular and atomic ions. In time-of-flight SIMS, the incident ion beam is pulsed, and the secondary ion mass-to-charge ratio (m/z), and hence its identity, is determined by the time it takes these sec-

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ondary ions to reach the ion detector. Imaging time-of-flight SIMS can be used to map the chemical composition of cell membranes. For example, using an ion beam with a diameter of 200 nm, Ostrowski *et al*. were able to resolve the heterogeneous distribution of lipids at highly curved intercellular fusion pores with a spatial resolution of about 250 nm (*7*). An acyl chain fragment ($C_5H_9^+$), which is a secondary ion produced from most membrane lipids, served as a general membrane marker. The specific identity of the lipids was determined from molecular fragments of their chemically distinct phosphate head groups.

In the NanoSIMS imaging experiments reported by Kraft *et al.*, a tightly focused beam of Cs^+ ions scans the sample. The resulting secondary ions, which are primarily mono- and diatomic, are identified by a conventional high-resolution mass spectrometer (see the figure). Direct determination of chemical composition from these data is essentially impossible, and isotopic labeling must be used. Kraft *et al.* examined a phase-separating binary mixture of lipids. In this mixture, one lipid is labeled with ^{13}C, yielding $^{13}CH^-$ secondary ions, and the other lipid is labeled with ^{15}N, yielding $^{12}C^{15}N^-$ secondary ions (*1*). Simultaneous monitoring of signals due to both secondary ions, which provide unique signatures from each of the two lipid types, enables quantitative mapping of the membrane chemical composition with a spatial resolution of about 70 to 100 nm. This observation sits squarely in the current blind spot with respect to biomembrane structure imaging.

Kraft *et al.* have corroborated the NanoSIMS images of membrane phase separation against atomic force microscopy (AFM) images of the same samples. AFM has previously been used to image lateral structures in lipid membranes deposited onto flat substrates. For simple model membrane systems, AFM can reveal nanometer-scale domain patterns with striking clarity (*8*). Kraft *et al.* found precise agreement between the AFM and NanoSIMS images of membrane domain structures. However, the NanoSIMS image also provided chemical specificity, which is lacking in AFM and most other forms of microscopy.

In biomembrane imaging, knowledge of chemical composition is critically important. The existence of heterogeneous structures in membranes is not in doubt; it is their chemical composition that is hotly debated. Do protein interactions nucleate membrane domains rich in saturated lipids and cholesterol (rafts)? Or does lipid phase separation sort the proteins into raft domains? Perhaps both mechanisms occur; if so, then how many types of domains are there? These questions have proven very difficult to address with current imaging technologies. Even in simple binary mixtures, the two phases rarely consist of pure components; the entropic cost is too large. Precise determination of phase composition—for example, with NanoSIMS—can reveal the strength of the driving force for phase separation and is a key ingredient in developing a physical picture of how membrane organization is governed.

NanoSIMS may also be used to image membrane-associated proteins in model systems or in whole cell membranes. Antibodies or small-molecule binders labeled isotopically or atomically (for example, with fluorine) could be used to mark the proteins of interest and map their positions. The repertoire of distinguishable secondary ions allows for a full spectrum of "colors" to identify numerous different molecules. Although imaging mass spectrometry is still in its infancy, it is emerging as a powerful technique that uniquely accesses a strategic gap in our knowledge of cell membrane structure.

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IMMUNOLOGY

Restless T Cells Sniff and Go

How a key immunological regulator prevents autoimmune disease has been unclear. Live imaging shows that it may prevent immune cells from lingering too long in the lymph nodes.

Tomas Mustelin

A long-standing puzzle in immunology has found a dynamic new explanation—quite literally. On page 1972 of this issue (*1*), Schneider and colleagues uncover a new paradigm for how a protein expressed on the surface of T lymphocytes suppresses excessive immune responses and prevents autoimmune disease. It turns out that T cell motility is the key.

T cells normally transit rapidly through the lymph node (at velocities of up to 25 μm/min), while continuously adhering to other cells, scanning their surfaces for antigens, those molecules that stimulate an immune response (*2*, *3*). Most immunologists had not expected T cells to move as fast or act as deliberately in their search for antigen.

Foreign antigens, such as those expressed by microorganisms or transplanted organs, are ingested and digested by dendritic cells, the professional antigen-presenting cells of the immune system. These antigen-loaded cells travel to their regional lymph node, where they present short peptides derived from antigens on their surface in the context of major histocompatibility (MHC) molecules. Dendritic cells also present ligands, accessory molecules, and adhesion molecules that are surveyed by T cells. When recognized, this suite of molecules can cause a transiting T cell to change its locomotive behavior (*2*, *3*). It slows down, moves around more carefully, and eventually forms a conjugate with the cell that presents an antigen recognized by the T cell receptor (see the figure). This contact, which can last for several hours, develops a highly organized molecular architecture called the "immunological synapse" (*4*) that acts as a hub for intracellular signaling cascades. These signals are delicately balanced between a forward urge to initiate T cell activation leading to a full immune response and negative influences to abort the mission. Numerous cell surface glycoproteins and signaling molecules recruited by the immunological synapse serve these opposing functions (*5*).

One of the best-studied negative regulators of T cell activation is cytotoxic T lymphocyte antigen–4 (CTLA-4) (*6*), a cell surface receptor for the B7 (CD80/CD86) molecule on dendritic and other antigen-presenting cells. CTLA-4 is a 41- to 43-kD dimeric transmembrane glycoprotein, and a

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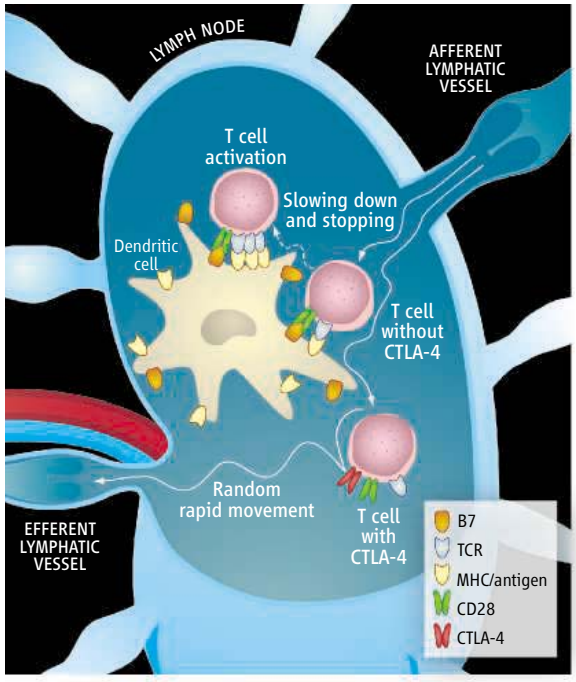
member of the immunoglobulin superfamily. Its expression is induced by T cell activation, and it is mostly found in intracellular reservoirs from which it can be rapidly exported to the cell surface. Mice lacking CTLA-4 develop a severe autoimmune phenotype with organ destruction (*7*, *8*), and polymorphisms in the human *CTLA-4* gene are associated with autoimmune diseases like type 1 diabetes (the immune system attacks pancreatic beta cells that make insulin) (*9*). However, how CTLA-4 suppresses excessive immune responses and autoimmunity has remained unclear. Proposed mechanisms include competition with the costimulatory T cell molecule CD28 for binding to B7, activation of the intracellular signaling molecules protein phosphatase 2A (*10*) and Src homology 2–containing protein tyrosine phosphatase SHP2 (*11*), release of the immunomodulatory enzyme indoleamine 2,3-dioxygenase (*12*), and less defined effects on membrane lipid rafts and the immunological synapse (*13*).



T cell motility in the lymph node. Under normal physiological conditions, a T lymphocyte transits through a lymph node and slows down to associate with cells that present antigens that it recognizes. This leads to T cell activation and an immune response. However, T cells that express high levels of CTLA-4 are more likely to continue to migrate until they exit the lymph node.

Schneider *et al*. now propose that CTLA-4 also regulates T cell motility by preventing T cells from slowing down to form long-lasting conjugates with antigen-presenting cells. Instead, T cells move on too rapidly to become fully activated, thus preventing the initiation of an immune response. Schneider *et al*. report that, whereas all T cells move around at similar speeds in the absence of antigen or T cell receptor ligands, only CTLA-4–null T cells slow down and form much longer lasting conjugates with antigen-presenting cells. In contrast, T cells expressing CTLA-4 continue their random migration at unchanged velocities when antigen is presented. This was observed in both cultured cells and in lymph nodes of live animals.

The role of CTLA-4 may be to ensure that a T cell hesitates when it encounters antigen, "sniffs" carefully, and quickly moves on unless the antigen is strong. Encounters with antigen-presenting cells that are too short can lead to an abortive immune response (*14*). However, in the absence of CTLA-4, T cells are too easily persuaded by brief encounters to slow down and forge an immunological synapse, even when the strength of T cell antigen receptor signaling is weaker than normally required for T cell activation. As a result, CTLA-4–null T cells respond to weak antigens (those that normally would not activate the T cell receptor), leading to autoimmunity. Indeed, polymorphisms in the promoter region of human *CTLA-4* that predispose to autoimmune disease are thought to reduce the expression of CTLA-4 on T cells. The high affinity of CTLA-4 for its B7 ligand has also been explored as an avenue for therapy: A soluble CTLA-4 protein can displace CD28 from B7 and thereby suppress T cell activation in intact animals (*15*).

So how does CTLA-4 affect T cell motility? T cell migration involves adhesion molecules [most notably, the integrin lymphocyte function–associated antigen-1 (LFA-1)], the actin cytoskeleton, and regulatory cellular signaling networks associated with both. Known as "inside-out" signaling, enhanced integrin-mediated cell adhesion results from intracellular signals that it receives from a T cell receptor that have been activated by an antigen. Paradoxically, CTLA-4 also increases integrin adhesive activity, but apparently by a different mechanism. Binding of CTLA-4 by B7 results in activation of Rap1 (*16*), a small GTP-binding protein that interacts with the integrin-associated adapter protein RAPL (*17*). RAPL in turn promotes surface expression of LFA-1 and, consequently, increases adhesion. It might seem counterintuitive that CTLA-4 would increase integrin adhesion activity and also promote motility, rather than cell-cell conjugates.

Clearly, pieces of the puzzle are still missing. T cell locomotion entails both adhesion and detachment coordinated by dynamic cytoskeletal events. In nonlymphoid cells, cell migration involves the rapid formation of adhesions at the leading edge of the cell with the extracellular substrate (focal adhesions) and their equally rapid disassembly at the cell's trailing end. Perhaps CTLA-4 stimulates both adhesion and detachment of T cells in a manner that keeps T cells moving and prevents them from executing the "stop moving" program and immunological synapse formation.

The work by Schneider *et al*. raises new questions that should stimulate research into the relationships between CTLA-4, T cell receptor and CD28 signals, T cell adhesion and migration, and the dynamics of the actin cytoskeleton. Are the previously proposed signaling mechanisms for CTLA-4 connected to the motility effect? T cell motility is a crucial factor in immunity, and a better understanding of the molecular underpinnings of how and when T cells move may enable the design of new treatments for autoimmune disease, including type 1 diabetes, rheumatoid arthritis, lupus, multiple sclerosis, and inflammatory bowel disease.

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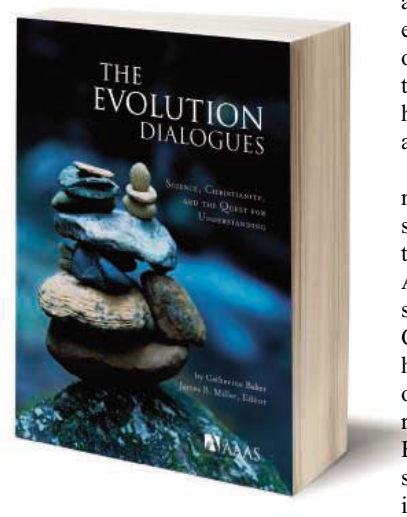
SCIENCE AND SOCIETY

"Evolution Dialogues" Seeks to Bridge Science and Religion

At a time when several leading scientists have written personal accounts of their belief in both God and the scientific method, a new AAAS book seeks to resolve some of the misunderstandings on both sides of the ongoing public debate over the teaching of evolution in the nation's public schools.

The book, "The Evolution Dialogues: Science, Christianity, and the Quest for Understanding," is a thoughtful look at both the development of evolutionary biology and the rich diversity of Christian responses to the theory. Written with the input of both scientists and theologians, it is intended to be an accessible, authoritative resource on evolutionary science that also can be used in schools and religious education classes.



One reason evolution remains a cultural flashpoint, the book notes in its prologue, is that there are "deep misunderstandings about what biological evolution is, what science itself is, and what views people of faith, especially Christians, have applied to their interpretations of the science. With this volume, AAAS seeks to correct some of those misunderstandings."

The book was written by Catherine Baker, edited by James B. Miller, and produced by AAAS's Dialogue on Science, Ethics, and Religion (DoSER). Connie Bertka, DoSER's director, said the book grew out of concerns among scientists and some religious leaders that intelligent design is being sold as an integration of science and religion, enticing even some members of mainstream religious communities to question evolution.

Proponents of intelligent design argue that there is empirical evidence in nature for the existence of an intelligent agent beyond nature, and they have tried to convince politicians and school officials that there is a scientific controversy over evolution. The new book details why any controversy is cultural and political, but not scientific.

Evolution remains one of the most substantiated theories in all of science, it notes, and serves as the essential framework for modern biology. The book discusses recent observations that have led to revisions in the theory since the time of Charles Darwin, including new views on why the giraffe's neck is long. But it emphasizes the underlying principles of evolution that continue to stand the test of time: all species, living and extinct, are related to each other, and the forms of life that populate the Earth have changed over eons and continue to change.

The book features a narrative about the personal dilemma of a fictional college student, Angela Rawlett, as she struggles to reconcile her Christian upbringing with her keen interest in biology. Her story is rooted in reality, according to Bertka. Students from some conservative Christian backgrounds sometimes approach biology professors with concerns that the study of evolution will conflict with their religious beliefs.

"Biology 101 teachers can cite cases like this," Bertka said.

In addition to its potential use in religious adult education programs, the new book also should have value in other educational settings such as history-of-science classes, seminaries, and community libraries. An array of distinguished reviewers, contacted by AAAS, found the book to be a useful, balanced treatment of the issues.

Jack Haught, a Georgetown University theologian, said the book "will prove to be very helpful to teachers and students of biology, especially where questions might arise about the scientific status of Darwin's theory and the religious implications of evolution." Haught also said the book "demonstrates how a religious understanding of the world need not be looked upon as an alternative to evolutionary science and vice versa."

Rodger Bybee, executive director of the nonprofit Biological Sciences Curriculum Study, said the book "will be an excellent, positive contribution to a contemporary understanding of evolution and religion."

To obtain "The Evolution Dialogues," and to learn about a free online study guide that can be used in church groups and other settings, see www.aaas.org/spp/dser.

—*Earl Lane*

SCIENCE DIPLOMACY

S&T Fellows Brave War to Stage GIS Conference

Marsha Goldberg and Fernando Echavarria

After months of difficult planning and preparation, an ambitious forum on geographic information systems (GIS) and sustainable urban development was days away from opening in Amman, Jordan. And then, in neighboring Lebanon, the war started.

For AAAS Diplomacy Fellow Marsha Goldberg and former Diplomacy Fellow Fernando Echavarria, it was a time of acute uncertainty. They had conceived and organized the conference—but would professionals from around the region still attend? Would it be best to postpone it or cancel it?

The conference started on schedule, and while the 4-day gathering was colored by tension and sadness arising from the conflict, it proved a powerful reminder that science and technology can help build constructive relations among the nations of the Middle East and North Africa and between the West and the Muslim world.

"These kinds of events are extremely important," keynote speaker Eduardo Lopez Moreno, chief of the Nairobi-based Global Urban Observatory for the U.N. Habitat, said in an interview. "When trying to respond to regions where there are some political tensions, this creates an excellent opportunity to use science and technology to build bridges and to use this as an example of the value of collaboration."

Goldberg offered a more personal reaction, putting her work on the conference in the context of her 2 years at the U.S. State Department. "This was the most satisfying activity of my Fellowship," she said.

Goldberg became a Diplomacy Fellow under the AAAS Science & Technology Policy Fellowship in September 2004. (After her fellowship ended in August, she joined the U.S. Millennium Challenge Corporation as a director of environment and social assessment.) Echavarria was a AAAS Diplomacy Fellow for a year beginning in 1997, and he currently serves in the Bureau of Oceans, Environment and Science at State.

Goldberg is an urban planner; Echavarria has been involved in past State Department efforts to promote the use of GIS. When Goldberg was assigned an office adjoining his, they developed the idea for the conference, then obtained seed funding and built a team that included other federal agencies and private companies.

In all, the GeoInformation for Sustainable Cities conference drew 50 planners, scholars, and government officials from 10 countries and regions in the Middle East and North Africa—Morocco, Tunisia, Egypt, Jordan, Libya, the West Bank and Gaza, the United Arab Emirates, Iraq, Kuwait, and Yemen. Fifteen of the participants were women; seven of the experts were from Libya, and seven more from Iraq.

GIS uses high-powered computer hardware and software along with mapping systems, satellite images, and socioeconomic data for a range of purposes, from tracking leaks in an urban water system to charting broad development and poverty patterns. These tools could be crucial in coming decades as hundreds of millions of people worldwide leave rural areas for makeshift urban settlements.

"This is where the sustainable development challenge will have to be met," Echavarria said. "How are we going to address the needs of the urban poor—clean water, health, transportation, housing, and other incredibly challenging problems?"

Moreno and others agreed that the conference would bring broad benefits and, ultimately, could promote democracy. "After the workshop, we can create a collaboration of people working together," he said. "When you have an extended support network, you feel more confident and more empowered to discuss issues you might not otherwise be able to."

Such events "can assist people living in the Middle East in dealing with the critical and extremely complex challenges that characterize the region in the political, social, and economical realms," said Nidal Saliba, GIS manager for the Water Authority of Jordan. That, in turn, promotes the "enhancement of people's everyday lives."

Over more than three decades, the AAAS S&T Policy Fellowships have brought scientists and engineers into public service in a variety of agencies and in Congress. The fellowships are designed to nurture links between federal decision-makers and scientific professionals to support public policy that benefits the well-being of the nation and the world.

SCIENCE TRAINING

An Energy Infusion for Global Career Outreach

Over the course of this fall, ScienceCareers.org's Garth Fowler will travel 8200 miles, crisscrossing time zones to attend programs in nine different U.S. cities. *Science* International's Seema Sharma, his European counterpart, is scheduled to attend events in London, Paris, Stockholm, and Manchester—all before 2007.

The schedule is rigorous, but with the training and retention of scientists and engineers a top global priority, *Science* and AAAS have moved in recent months to add energy to their career-outreach program. Though the programs have long been seen as among the world's best, Fowler, Sharma, and their colleagues are busy planning, implementing, and evaluating the programs to make them even better.

"With great people on two continents, the full support of AAAS, and synergy between our outreach events and our editorial products, we are prepared to make an even larger positive impact on the careers of today's and tomorrow's scientists and engineers," said Jim Austin, the editor of ScienceCareers.org.

Late last year, a decade after their first career resources went online, *Science* and AAAS moved to dramatically update their programs, consolidating powerful recruitment and job-search features with *Science*'s Next Wave, GrantsNet, the Minority Scientists Network, and other features—all at one fresh-looking, easy-to-navigate site. At ScienceCareers.org, users now are offered free access to over 2000 job postings, along with career advice articles, graduate school listings, and funding opportunities.

"ScienceCareers.org's goal is to increase awareness of the job opportunities and resources available for scientists," Sharma said. "We offer fundamental, practical advice on how to be successful—whether you do benchwork, clinical studies, public policy, or something else."

In addition to online resources, ScienceCareers.org has teamed up with nonprofit organizations like the National Postdoctoral Association and the University Consortium, along with universities and private institutions such as New York University, to provide essential career-advancement skills and resources.

For example, this fall AAAS and its partners are co-sponsoring the Scientific Management Course for Postdoctoral Fellows, a laboratory management program geared to young scientists.

Many career-development experts and counselors at academic and research institutions believe that AAAS career-development programs are instrumental in training the next generation of scientific investigators.

"These courses are in high demand and springing up all over the country at various institutions and at professional scientific society meetings," Fowler said.

—*Benjamin Somers*

SCIENCE EDUCATION

S&E Capacity Center Gets Strong Reviews

Two years after it was founded, an ambitious AAAS effort to help colleges and universities more effectively recruit and retain science and engineering students is showing positive results.

The AAAS Center for Advancing Science and Engineering Capacity took on eight new clients, disseminated its research findings at conferences and workshops and in publications, and raised more than a half-million dollars in revenue in the year ending 30 June 2006, center Director Daryl Chubin said in a report to The Alfred P. Sloan Foundation.

The Foundation awarded AAAS a 3-year, $400,000 grant in 2004 to underwrite the new center's plan to offer consulting services to individual universities and colleges seeking to increase the participation of U.S. students, especially women and underrepresented minorities, in science and engineering careers. The goal has been to make the center self-supporting after 3 years (see www.aaascapacity.org).

"We understand the demographic, financial, and legal pressures that universities must balance," Chubin said. "The problems that emerge from imbalances require tailor-made solutions. 'Off-the-shelf' won't do. This is about local context, policies, and practices. The need for help from the outside is clear—and growing."

Ted Greenwood, the Sloan program director who oversees the AAAS grant, said the gains in the center's second year bode well for the future. "We are hopeful that they have now become sufficiently visible and developed a sufficiently positive reputation that their client base will continue to grow over time," he said.

In other education news, the U.S. National Science Foundation (NSF) has awarded AAAS Education and Human Resources a grant of $973,572 to organize and co-sponsor the 2007, 2008, and 2009 conferences for awardees of the NSF's Historically Black Colleges and Universities Undergraduate Program. The program provides funds to broaden participation in the nation's science, technology, engineering, and mathematics workforce. About 800 people—more than half of them students—are expected to attend each annual conference.

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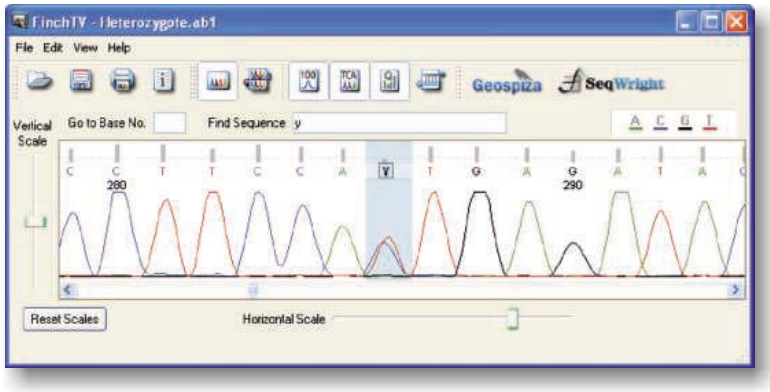

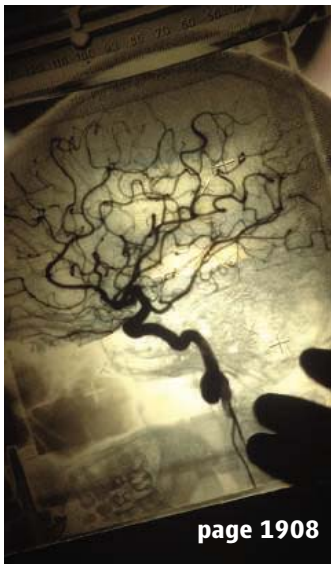
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INTRODUCTION

Genomic Tales

OUR ORGANS TELL STORIES. A PATHOLOGIST, FOR EXAMPLE, CAN LOOK AT A LUNG and recognize a lifetime of toiling in a mine. Our genes tell stories, too. By comparing the genomic sequences of an ever-increasing number of organisms, we are now uncovering how our bodies came to be the way they are. Evolution, it seems, is a tale of détente: The need to adapt to changing environments is in a tug of war with the demand for precisely functioning biological machinery. The stories presented in the special section (and the graphic, p. 1912) emphasize different facets of this complex saga. They are not just historical lessons; they have implications for understanding disease mechanisms as well as basic physiology.

When it comes to the story of the human brain, we are still stuck on the preface, Pennisi explains in a News story (p. 1908). Researchers are turning to comparative genomics to identify the main genetic characters that helped differentiate our brain from those of our primate cousins. They are finding evidence of positive selection for genes that are key to the size and complexity of the cortex, as well as provocative changes in gene copy number and expression.

Fernald, in discussing the evolution of the eye (p. 1914), notes that despite the seeming diversity of eyes, there has been a lot of reinvention and reuse. Nothing is wasted. Duplication of an ancestral opsin gene resulted in photoreceptor cells with very different light sensitivities and capacities. Organisms appear to have capitalized on a variety of excess proteins by diverting them into lens production; in some cases, lens proteins still have multiple functions.

Olson (p. 1922) emphasizes the staying power and flexibility of the regulatory networks that coordinate gene expression in the heart. A primordial regulatory network of five transcription factors has been conserved for at least 500 million years. Complexity has been generated by pulling in other genes and networks.

Animals have become morphologically sophisticated thanks to Hox genes. They were intimately involved in the evolution of bilateral symmetry, and changes in the number and expression of members of the Hox gene family may underlie much of life's current morphological diversity (Lemons and McGinnis, p. 1918).

Three articles at *Science*'s STKE highlight the regulatory mechanisms by which gene expression profiles pave the way for complex structures. Bondos covers how Wnt signaling and Hox gene–expression networks work together to specify cell fate and tissue formation in *Drosophila* and *Caenorhabditis elegans*. Shamovsky and Nudler discuss the role of a large noncoding RNA in vertebrate organogenesis. Lamont and Childs discuss arterial versus venous specification.

Genome issues always have more exciting stories than can fit under one theme, and this issue is no exception. Out of a growing foundation of insights into the genetic infrastructure needed to build our bodies are coming new ways to diagnose and fight diseases. See *Science* Careers for vignettes of young researchers tackling these problems.

Like the tales of Scheherazade, one story leads into the next, and we can only look forward to the upcoming chapters.

–BARBARA R. JASNY, ELIZABETH PENNISI, JOHN TRAVIS

Building the Body From Genes

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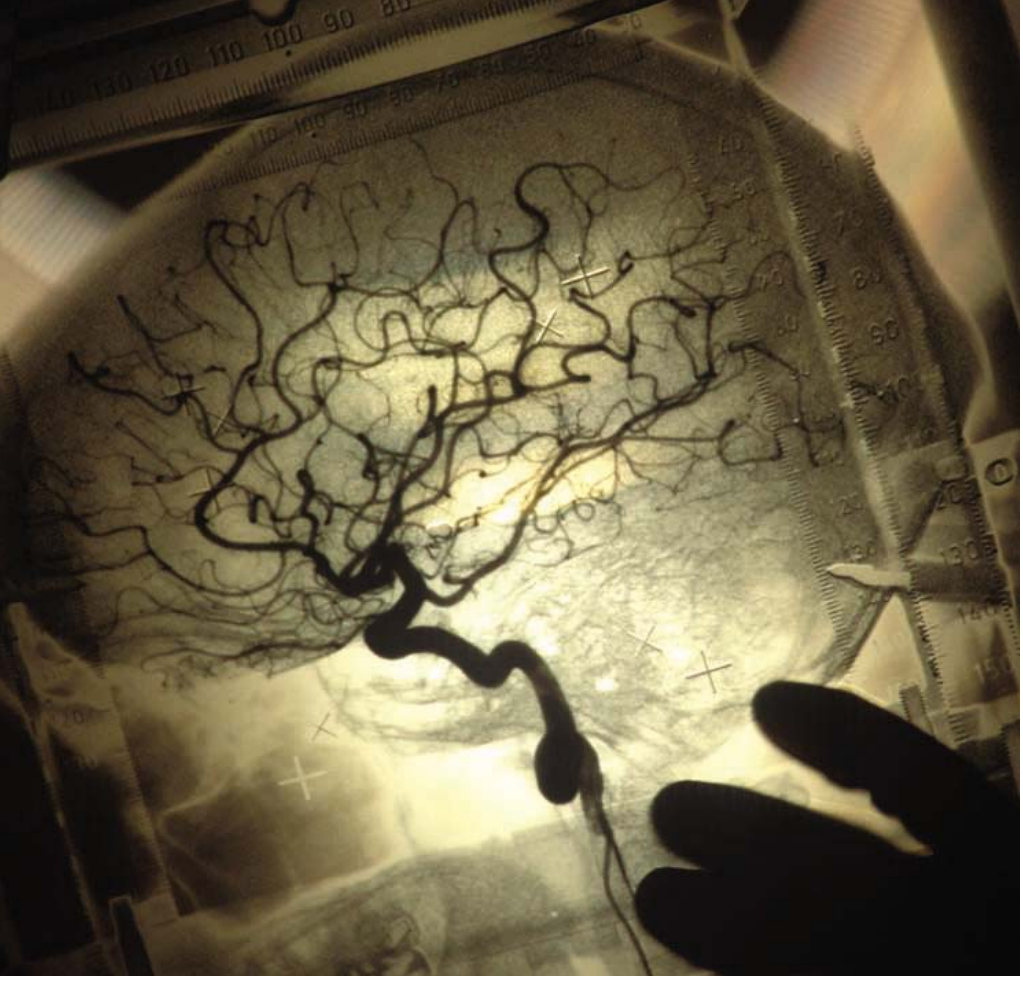

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NEWS

Mining the Molecules That Made Our Mind

By comparing the human genome with those of other species, researchers are finding many genes potentially related to brain evolution—but no one is sure which ones helped shape the uniquely human brain

IN THE 17 AUGUST *TORONTO SUN*, THE headline trumpeted "A single gene led to humans. …" *The Independent* in the United Kingdom declared "Revealed: the gene that gave us bigger brains." And in Minnesota, newspaper readers were greeted with "Scientists ID genes that make humans smarter than chimps." All three stories announced the discovery of a brain-related genetic difference between chimps and humans, and in all three, the newspapers got carried away.

True, the gene featured, called *HAR1F*, is active in the right place at the right time during brain development to have spurred the expansion of the cerebral cortex, the center for higher cognition in people. *HAR1F*, which stands for "human accelerated region 1 forward" gene, also shows signs of rapid change since humans and chimps went their separate ways, suggesting that this gene conferred a survival advantage to our ancestors (*Science*NOW, 16 August, sciencenow.sciencemag.org/cgi/content/full/2006/816/2).

Yet *HAR1F* is only one of about 10 genes to emerge in the past 4 years as potentially key to the evolution of the uniquely skilled human brain. And these discoveries are but the beginning chapters of an epic evolutionary story that we are just starting to read. With the genomes of dozens of species in hand, including human, chimp, and rhesus macaque, as well as powerful bioinformatics methods for comparing and analyzing all this DNA, research into the molecular basis of human evolution has exploded. Scientists using multiple comparative genomic strategies have uncovered hundreds of genes that provide tantalizing clues about hominid evolution. And some of the most provocative finds pertain to brain evolution.

A few researchers, for example, are delving deep into the tree of life, uncovering genetic evidence about when a central nervous system first began to fire from analyses of the genes of simple organisms such as jellyfish. But the scientists grabbing the headlines are the ones finding brain genes that have changed rapidly, duplicated, merged, or boosted their expression since our lineage split from that of chimps. Such genes are prime candidates for crafting the modern human brain.

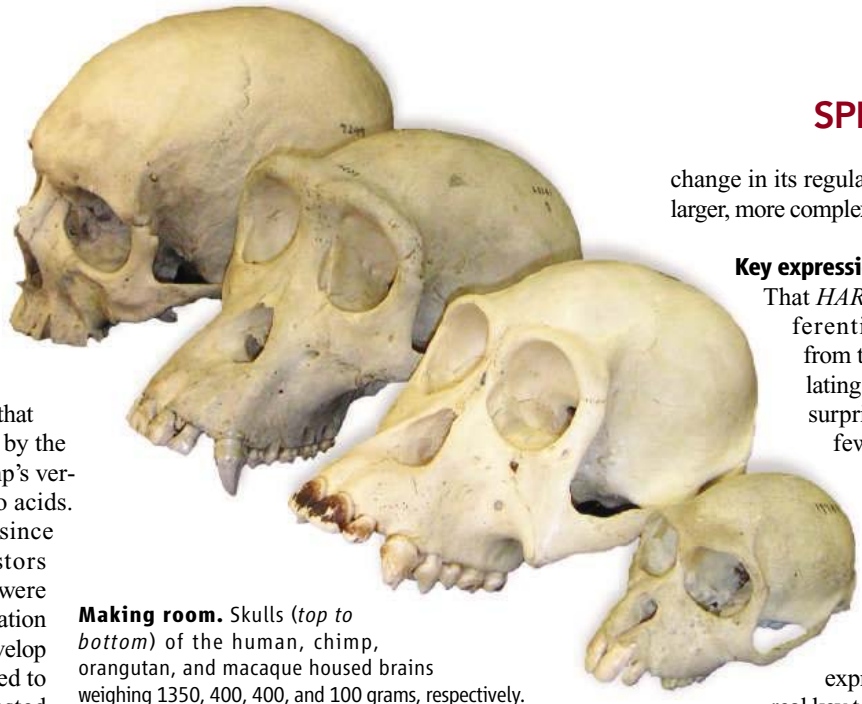
Many scientists find the potential to understand ourselves irresistible. A rush of comparative genomics results about human evolution are being presented at meetings, and publications are starting to stream out. "Everyone is jumping on the bandwagon," says Bruce Lahn, a human geneticist at the University of Chicago, Illinois. Progress at identifying the DNA tweaks that distinguish people from other species should be swift. "Within a year or two, we will have a comprehensive list of genome changes to start looking at," says Christopher A. Walsh, a neuroscientist at Harvard Medical School in Boston.

Emphasize "start." Despite what the headlines imply, genomic data so far offer provocative clues rather than direct answers to what caused humans to branch out from the primate tree to become walking, talking, creative beings. Tying genomic events such as a gene duplication to human evolution is a challenge, because often researchers know little about what their candidate genes do. To make a concrete connection between a genetic change and the evolution of the human brain "is a much slower process," says Walsh.

Positive results

Philosophers and scientists alike have long sought to understand what makes humans unique in the animal kingdom. Among other differences, our brain is three times the size of a chimp's, with a multilayered cortex capable of doing calculus or writing plays. Much of the explanation for our braininess is encoded in the genome, but with 20,000 or so genes to choose from, geneticists have only just begun to come up with effective search strategies to highlight the crucial ones.

One method is to seek genes that natural selection has favored in humans but not in our close cousins, the chimps. Genes that have experienced such positive selection have evolved more quickly than the background rate of evolution. Researchers can spot these speedy evolvers by comparing genes in humans and



Making room. Skulls (*top to bottom*) of the human, chimp, orangutan, and macaque housed brains weighing 1350, 400, 400, and 100 grams, respectively.

other species. For example, a gene called *FOXP2* is mutated in a family with a severe language disorder; 6 years ago, a team led by Svante Pääbo at the Max Planck Institute (MPI) for Evolutionary Anthropology in Leipzig, Germany, found that the human protein encoded by the gene differed from the chimp's version in two of its 715 amino acids. Given the amount of time since chimp and human ancestors diverged, no such changes were expected. The protein's alteration may have helped humans develop the fine motor control needed to mouth words, Pääbo suggested (*Science*NOW, 14 August 2002, sciencenow.sciencemag.org/cgi/content/full/2002/814/2).

Pääbo looked for evidence of positive selection on one gene, but Lahn and his colleagues have taken a broader sweep across the genome. They examined 214 genes involved in brain disorders or active only in the brain, comparing humans and macaques and, in a separate analysis, mice and rats. For each pair, the researchers counted both the changes in a gene's bases that made no difference to the encoded protein—considered the background rate of evolution—and the changes that altered an amino acid. The higher the proportion of protein-altering changes, the faster the gene had evolved.

Overall, Lahn's team found that evolution in the primate genes was trotting along about 37% faster than in the rodent genes. Among just the two primates, human brain genes, particularly those involved in development, were the sprinters, outpacing the number of changes in the equivalent genes in the macaque. Two genes showed particularly strong evidence of selection: *ASPM* and *microcephalin*, each of which underlie microcephaly, a genetic disorder that results in a diminutive brain, Lahn and his colleagues reported at the end of 2004.

The "single gene" heralded last month by the *Toronto Sun* emerged from an even broader search for positive selection—one that covered entire genomes. David Haussler and Katherine Pollard, both bioinformaticists at the University of California (UC), Santa Cruz, and their colleagues developed a sophisticated computer program that matched up the sequenced genomes of multiple species, including chimp, human, rodent, dog, and chicken. The scan, reported online 16 August in *Nature,* picked out 49 regions in which most genomes shared the same sequence but the human DNA was considerably different, indicating it had changed quite a bit since roughly 6 million years ago when our ancestors split off from other primates.

HAR1F, for example, is part of a DNA sequence that, compared with the other species, has experienced 18 base changes over its 118-base stretch; less than one such base change would be expected over those 6 million years based on the accepted mutation rate for human DNA.

HAR1F codes for an RNA that is never translated into a protein. UC Santa Cruz cell biologist Sofie Salama, working with Pierre Vanderhaegen, a neuroscientist at the University of Brussels in Belgium, has found that the gene is very active in the developing brains of 2-month- to 5-month-old human embryos. The RNA exists in cells that organize the human cerebral cortex into layers, and because RNA can play a role in gene regulation, Pollard and her colleagues suspect that *HAR1F*'s RNA helps control the production of proteins involved in cortex development and that a change in its regulatory abilities prompted a larger, more complex cortex.

"The challenge is to link the evidence of positive selection to brain function."

—Svante Pääbo, Max Planck Institute for Evolutionary Anthropology

Key expression

That *HAR1F*'s possible role in differentiating the human brain from the chimp's involves regulating other genes should not be surprising. There are relatively few differences in the proteins encoded by the two species' genes—*FOXP2* being one exception—and researchers have long suspected that changes in where, when, and how much a gene is expressed may instead be the real key to our uniqueness.

To understand the evolutionary importance of changes in gene activity, some genomicists have simply looked for genes that are more active in one species than in another. Others have sought out extra copies of genes and other DNA that might also affect gene expression.

Pääbo pioneered the former approach in 2002 when he, Wolfgang Enard, also of the MPI for Evolutionary Anthropology, and their colleagues compared the overall amount of messenger RNA (mRNA) produced in various human, chimp, orangutan, and macaque tissues, including gray matter. "This was the first attempt to use high-throughput technology to address this very important issue," says Xun Gu, a molecular evolutionist at Iowa State University in Ames.

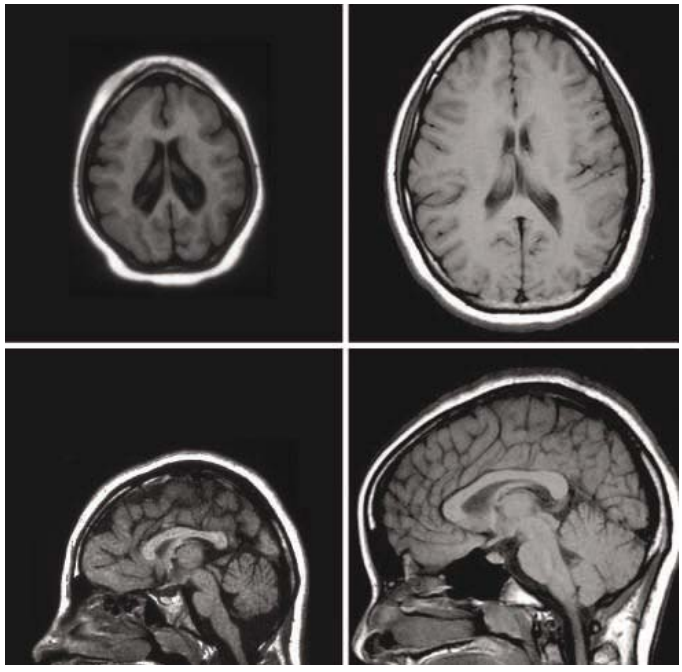
Pääbo's crew used a microarray to detect the mRNA concentration of 12,000 genes. Overall, Pääbo and his colleagues found that genes in the brain tend to have undergone more changes in expression—increases or decreases relative to the chimp—compared with genes in other organs since the two lineages diverged. But they saw little such gene-expression variation between brain and other organs when comparing the chimp with other primates. The result suggested that altered gene activity played a role in distinguishing the human brain from those of its cousins, Pääbo and his colleagues reported (*Science*, 12 April 2002, pp. 233, 340).

Gu's team later took a closer look at Pääbo's data and found a trend in the human versus chimp gene-expression changes in brain. The human brain genes typically had greater activity than their chimp counterparts. "We demonstrated that evolutionary changes may be mainly caused by a set of genes with

CREDIT: C. A. WALSH *ET AL.*, *PLoS BIOLOGY* 3, 50 (2004)

increased rather than decreased expression," says Gu.

Mario Cáceres of the Salk Institute for Biological Studies in San Diego, California, and his colleagues have reached a similar conclusion. They've used mRNA assays to compare gene expression in the cerebral cortex of humans, chimps, and macaques, finding 91 genes that had changed their activity level since chimps and humans went their separate ways evolutionarily. About 80 of those became more active in the human brain, they noted in 2003.



Genetic reduction. Several genes implicated in human evolution are linked to microcephaly, in which the cortex is smaller (*left panels*) compared to the normal brain (*right panels*).

Double the dose

None of these original expression studies tried to pin down the cause of the increased gene activity. But studies by James Sikela, a genome scientist at the University of Colorado Health Sciences Center in Aurora, Evan Eichler of the University of Washington, Seattle, and others offer one potential explanation: The human genome has plenty of extra copies of genes and other DNA sequences.

In 2004, Sikela, Jonathan Pollack of Stanford University in Palo Alto, California, and their colleagues did a pioneering full-genome scan of five primates, including humans, and found 1005 genes that had increased or decreased in number in one of the species after it split off from the ancestral primate tree. Of those, 134 had undergone duplication in the human lineage.

The next year, Eichler and his colleagues completed genomewide catalogs in humans and chimps of so-called segmental duplications, pieces of copied DNA that range in size from a few thousand base pairs to large sections of chromosomes. Eichler's group found that the genomes have separate duplication histories. Overall, duplicated segments in the chimp outnumber those in human, but in humans, there's a greater variety. More genes appear multiple times in our DNA than in chimp DNA. By one count, humans have extra copies of 177 full and partial genes. Each of those genes is a candidate for having altered expression patterns, and Eichler has been investigating the function and activity of several duplicated regions.

The availability of multiple copies of a gene provides more than just a way for a gene to produce additional amounts of its protein or RNA. These extra copies are material with which evolution can play, perhaps dedicating the activity of one copy of a gene to a subset of cells, for example. "The likelihood of innovation is much higher," says Eichler.

In some cases, such innovation could come from parts of a gene that proliferate instead of a whole gene undergoing duplication. Sikela, Magdalena Popesco, a molecular geneticist at the University of Colorado Health Sciences Center, and their colleagues have recently discovered a stretch of DNA, consisting of just two exons from a gene, that has increased from a single copy in mice to 212 copies in humans and may have evolved crucial new functions in the process.

Sikela's previous work had identified 134 genes that duplicated primarily after human ancestors split off from other primates. Popesco, Sikela, Gerald Wyckoff of the University of Missouri, Kansas City, and their colleagues compared the sequences of these genes in primates, mice, and rats, and one gene in particular stood out: *MGC8902*. Humans have 49 copies of this gene, whereas chimps have 10 and macaques have four, the group reported in the 1 September issue of *Science* (p. 1304).

No one knows exactly what the gene does, but a closer look revealed that it primarily consists of six copies of a two-exon segment that encodes a protein domain—a peptide that has a particular fold or twist—called DUF1220. This domain also has no known function, but the DNA for it is sprinkled liberally throughout the human genome, with the two-exon segment showing up in about two dozen different genes. These genes are active throughout the body, but Popesco notes that they are particularly busy in neurons in the cortex, suggesting that the domain is important in the brain. "The work highlights the potential importance of duplications in the emergence of novel genes within the hominoid lineage," says Eichler.

Another intriguing human gene apparently arose when part of one gene replaced part of another. While comparing the human and chimp genome, glycobiologist Ajit Varki of UC San Diego had noticed that the front end of a gene called *SIGLEC-11* was quite different between the two species, whereas the back end was virtually identical. The front end of the human version turned out to come from a gene called *SIGLEC-16*, which appeared as a duplicated copy of *SIGLEC-11* about 15 million years ago. *SIGLEC-16* is now dysfunctional in both the human and the chimp, suggesting that it lost function before the two lineages split.

However, at some point in hominid evolution, part of *SIGLEC-16* must have replaced the front part of the DNA of *SIGLEC-11*, creating a "brand-new, 'human specific' protein," says Varki. The gene for this protein now turns on in the human brain, specifically in microglia, cells known to be important to the growth of nerve cells (*Science*, 9 September 2005, p. 1693). The finding is "tantalizing, but of uncertain significance," Varki points out.

Finding function

Varki's measured summary of the *SIGLEC-11* story would be apt for many of these headline-grabbing genes. Whether it's *FOXP2*, *HAR1F*, or the DUF1220 domain, "there's a tendency for people to think this [gene] is it"—the explanation for why people are unique, says Pääbo. For his part, Varki warns that the quest to understand human evolution is too brain-centric. "We are also defined by differences in our reproductive, musculoskeletal, and immune systems, as well as by our skin," he points out.

Moreover, almost every gene linked to human brain evolution comes with questions

CREDIT: J. BOND *ET AL.*, *NATURE GENETICS* **37**, 353 (2005)

about the strategies used to establish the connection. For example, some scientists are not convinced that the methods used to detect positive selection in a gene are reliable or that the results will hold up as more genome sequences are added to these analyses. Genes that appear to change rapidly may still fall within the range of normal variation, for example. Moreover, rapid change in a gene or extra copies "does not mean that these genes are [all] important during the evolution of the human brain," says Gu.

Some scientists also question the relevance of certain findings, such as a protein-coding gene's mRNA being produced in a brain region. After all, not all mRNAs are translated into proteins. "I wouldn't put much stock in claims about genes being expressed or not expressed in the brain unless there's direct experimental evidence" that the genes' proteins are actually made, says Todd Preuss, a neuroscientist at Emory University in Atlanta, Georgia.

Eichler illustrates the challenges the field faces when he complains about spending the past 5 years trying in vain to figure out the role of a promising family of genes that emerged from his survey of segmental duplications. The family is in one of the most rapidly evolving duplicates. The genes are about 12 million years old and have undergone rapid evolution in humans, chimps, and gorillas. But the gene doesn't exist in mice, and the human copies and their surrounding DNA are too similar to track individually in disease studies.

Pääbo shares Eichler's frustration at the field being unable to move more swiftly beyond highlighting candidate brain-evolution genes. "It's getting a little stale," he admits, "to say, 'I have another case of positive selection.' The challenge is to link the evidence of positive selection to brain function."

That may be starting to happen in a few select cases. Take the microcephaly genes, *ASPM* and others, which may provide clues about how the human cortex got so big. Wieland Huttner of the MPI of Molecular Cell Biology and Genetics in Dresden has demonstrated how the amount of ASPM affects brain growth in mouse embryos. He studied mouse embryonic neuroepithelial cells, the stem cells that give rise to neurons. The longer these stem cells remain undifferentiated, the more they divide and the more neurons that ultimately form.

In anticipation of cell division, ASPM concentrates at opposite ends of the cell and helps organize the microtubules that pull duplicated chromosomes apart. When Huttner reduced the cell's cache of ASPM, cells no longer divided symmetrically. Instead of forming two daughter stem cells, one of those "daughters" specialized as a neuron, short-circuiting the expansion of the cortex, his team reported in the 5 July *Proceedings of the National Academy of Sciences*.

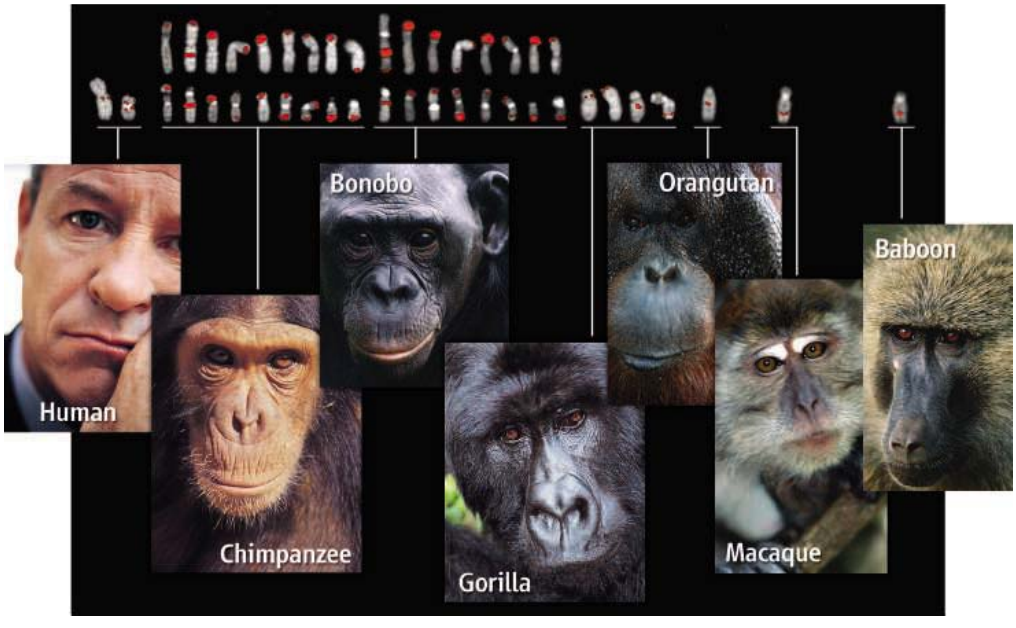
Researchers have found that two other genes associated with human microcephaly are active during cell division as well. Last year, Jacquelyn Bond of the University of Leeds in the U.K., C. Geoffrey Woods of the University of Cambridge, and their colleagues used mice to show that cyclin-dependent kinase 5 regulatory protein (CDK5RAP2) and centromere-associated protein J (CENPJ) are active in the same cells affected by ASPM, the embryonic neuroepithelial cells of the frontal cortex. Both the CENPJ and CDK5RAP2 proteins colocate with ASPM during cell division, Bond, Woods, and their colleagues reported. Earlier this year, Lahn's team showed that the genes for these two proteins have been evolving rapidly in the human lineage, similar to *ASPM*.

Another somewhat developed scenario for human brain evolution centers on the DNA that regulates a gene called *prodynorphin* (*PDYN*). The protein encoded by the gene is a precursor for opiate compounds important in perception, pain, social behavior, and learning and memory. In rats, for example, increased production of prodynorphin in the brain translates into higher pain thresholds. The gene's activity is under the influence of a 68-base DNA sequence. Greg Wray, an evolutionary biologist at Duke University in Durham, North Carolina, and his colleagues have found that humans have up to four copies of this regulatory stretch, whereas monkeys and other great apes only have a single copy. Moreover, the researchers have found five base changes in this regulatory DNA that have occurred since the split between chimps and humans, a sign of accelerated evolution.

In the lab, nerve cells with the chimp version of the regulatory sequence make less prodynorphin, Wray's team reported in the December 2005 *PLoS Biology*. That same regulatory DNA from the rhesus macaque, gorilla, and bonobo also failed to stimulate adequate prodynorphin mRNA production in human nerve cells. It seems that "humans have evolved to make more of this key brain peptide," Wray concludes. His team is now reconstructing the ancestral sequence of this piece of DNA.

The prodynorphin example is one of the most advanced, but even this evolutionary story is unfinished, because no one knows exactly what effect the extra prodynorphin has in the human brain, says Wray. And most other examples are in even earlier stages. "In virtually all cases, the link of genes or genomic patterns with human brain evolution is only tentative and based on suggestive evidence," says Lahn. "The situation may not change anytime soon due to the complexity of the questions and because we can't redo the experiment that evolution did in many millions of years." Headline writers, pay heed.

–ELIZABETH PENNISI



To each their own. Speciation among primates was helped by the duplication of DNA segments that helped differentiate genomes. Stained chromosomes reveal that whereas macaques and baboons have one copy of this segment (red), humans have four, and chimps and bonobos have hundreds.

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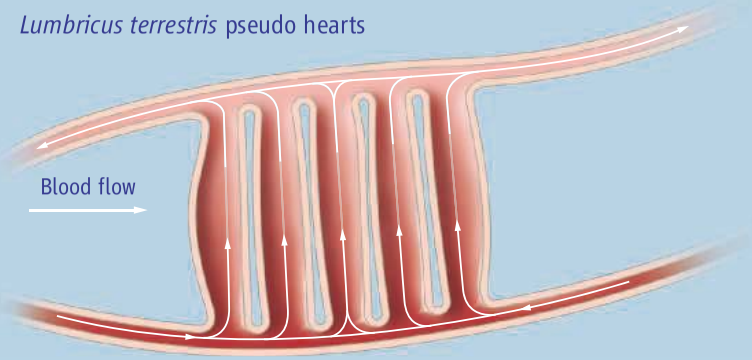
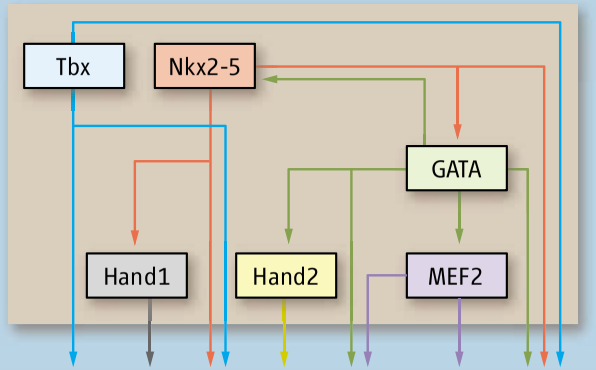
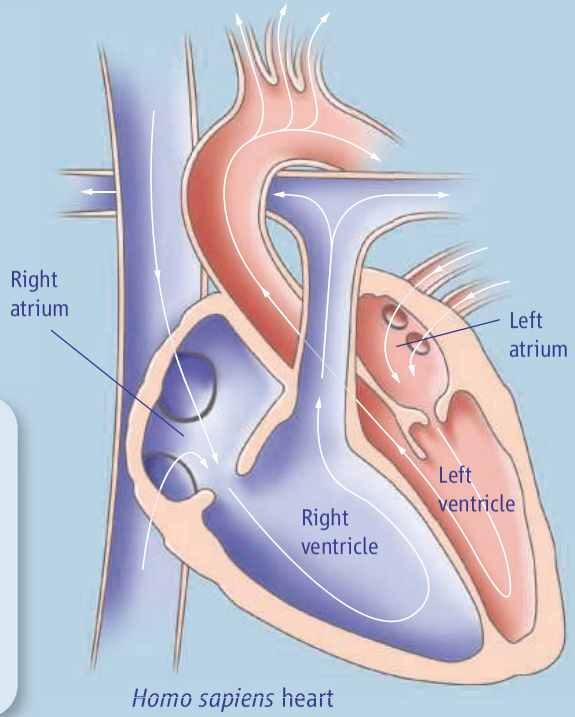
Lumbricus terrestris pseudo hearts



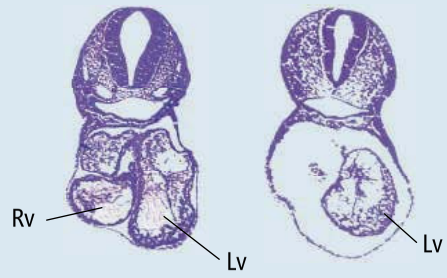


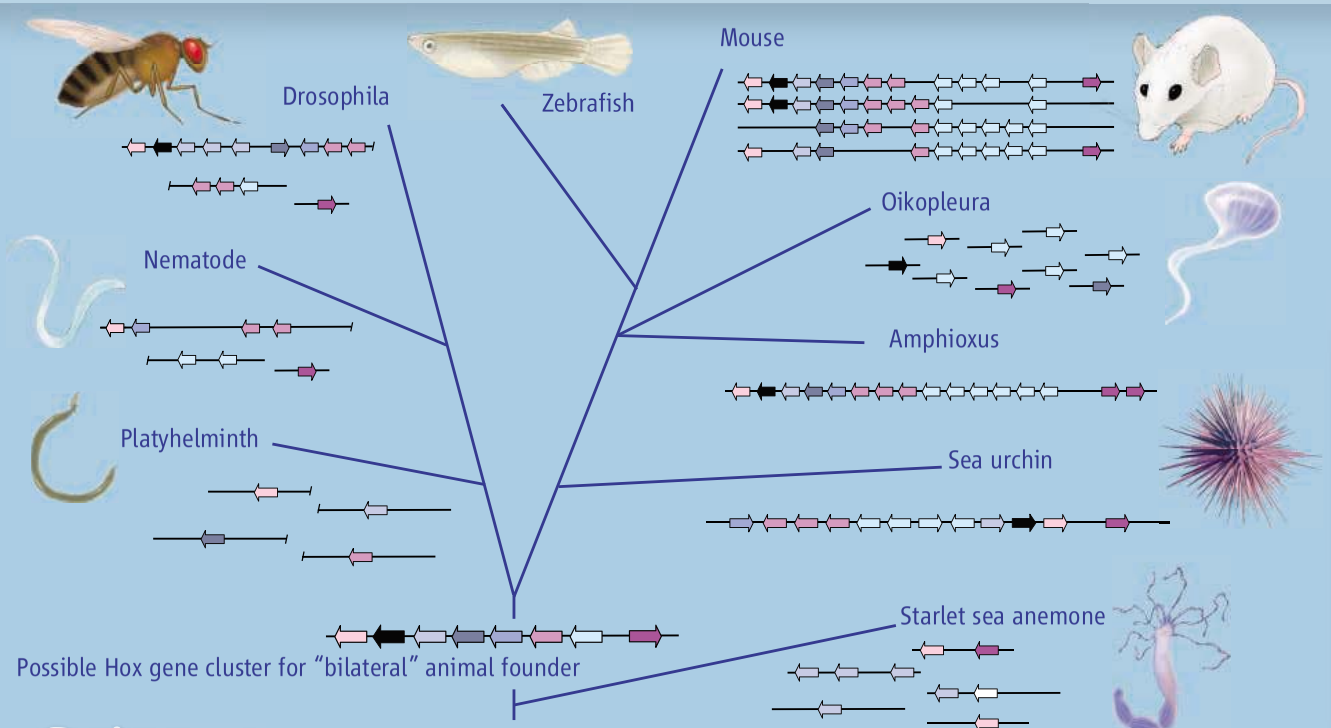


Homo sapiens heart

ALL HEARTS share a common function—to pump fluid carrying oxygen and nutrients through the body. A core group of transcription factors that connect signaling pathways with genes for heart muscle growth, patterning, and contractility have been conserved for roughly 500 million years. The flow chart shows a simplified version for vertebrates. Complexity has arisen during evolution through such mechanisms as gene duplication and incorporation of additional networks of interacting genes.

MEF2 is the most ancient factor regulating heart formation. In mice, the right ventricle of the heart does not develop if MEF2C is inactivated, as shown in these histologic sections of a wild-type (left) and mutant (right) embryo. Right ventricle (Rv), left ventricle (Lv).

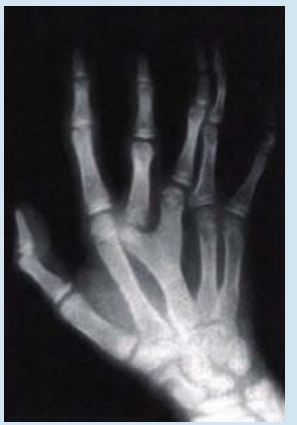




Possible Hox gene cluster for "bilateral" animal founder

MEMBERS OF THE HOX GENE FAMILY are responsible for controlling pattern formation—the formation of specific tissues and organs at specific locations. During the course of evolution, there have been expansions, losses, and fragmentation of clusters of Hox genes that are found on chromosomes, as exemplified in the phylogenetic tree (shown at left).

MANY MUTATIONS in the Hox gene family are lethal. In humans, expansion of a polyalanine repeat in *Hoxd13* triggers polydactyly (an increased number of digits on the hands or feet) when heterozygous and syndactyly (a failure of the digits to separate) when homozygous.



Science

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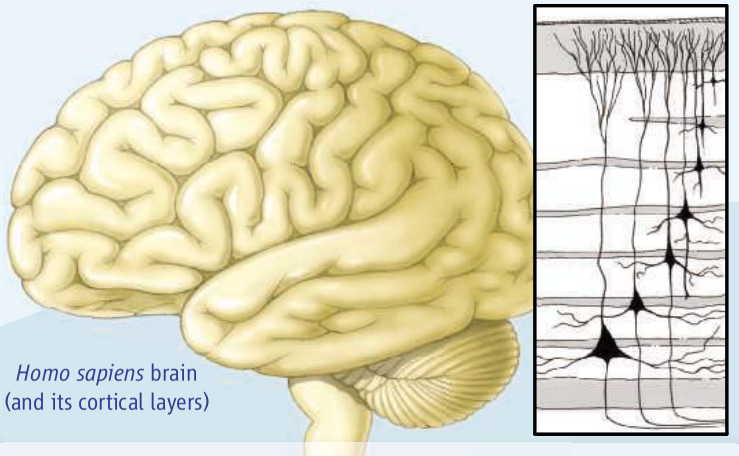
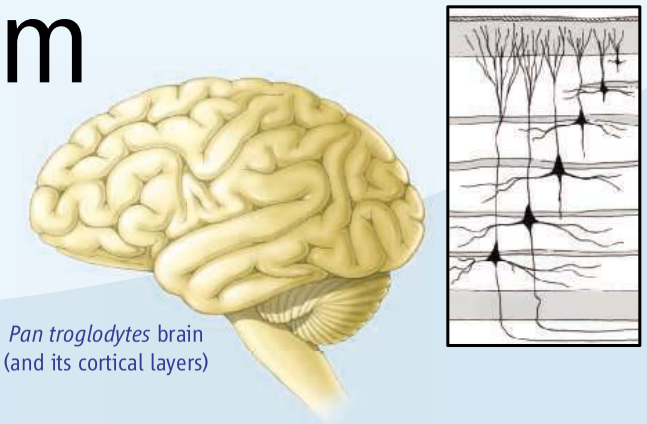
The Evolution of Function & Form

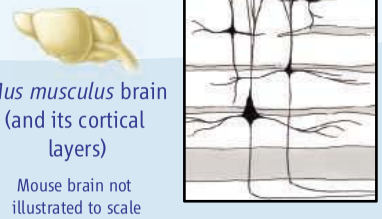
THE HUMAN BRAIN is dramatically expanded compared to that of a mouse or a chimp, both in overall size and in the complexity of its cortical layers. Scientists are beginning to identify genetic differences among these species that may account for our evolutionary jump in brain power, but no clear explanation is yet at hand.



Homo sapiens brain (and its cortical layers)



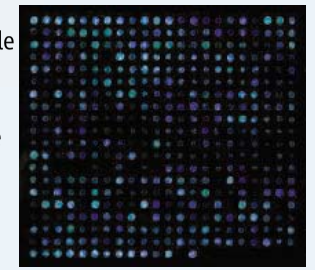
Pan troglodytes brain (and its cortical layers)



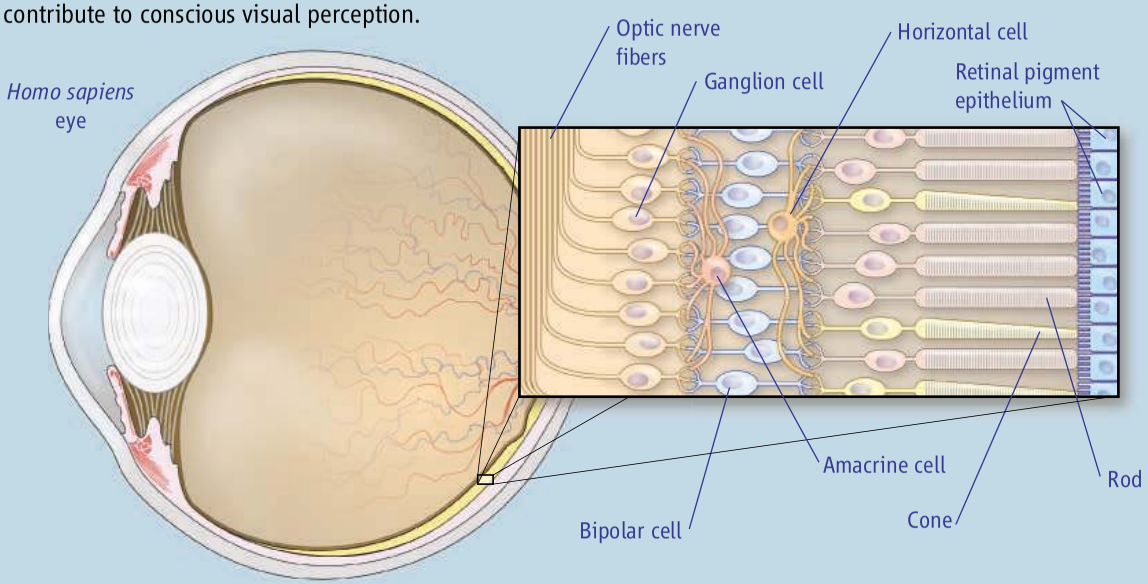
Mus musculus brain (and its cortical layers)

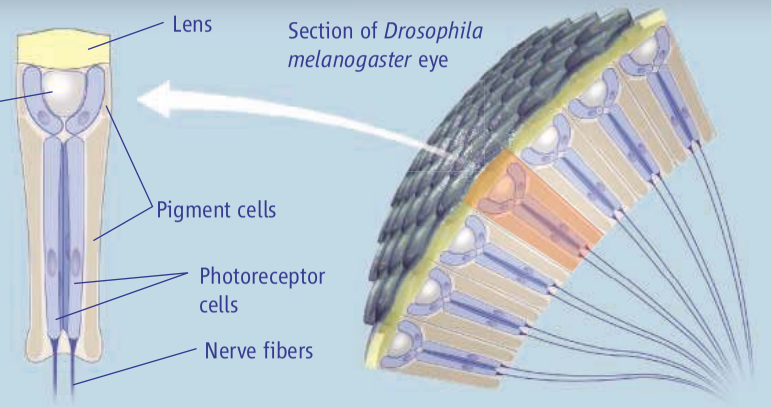
Mouse brain not illustrated to scale

MICROARRAYS, chiplike devices covered with DNA, are allowing the rapid comparison of whole genomes, illuminating genetic differences between species that may have given rise to the brain, heart, eye, and other bodily features. The ultimate evolutionary project would be to compare the human genome to that of a Neanderthal, which could shed light on why we survived and they didn't.



ANCESTRAL EYES probably had at least two kinds of light-detection systems; the evolution of vertebrate eyes resulted in primary use of one type, ciliary, leading to rods and cones, whereas invertebrate eyes evolved by use of the other, resulting in rhabdomeric receptors. However, some invertebrates contain bits of the vertebrate system, and functioning remnants of the invertebrate system can still be found in vertebrate eyes where they may contribute to conscious visual perception.





RHODOPSINS are expressed in vertebrate photoreceptor cells and act as light sensors in the pathway of visual perception. They consist of the protein opsin linked to 11-cis-retinal. Although expansion of the opsin family has allowed adaptation to different environments (and the evolution of color vision), the tandem duplications of the opsin gene that have arisen on the X chromosome in humans facilitate the homologous recombinations that result in color blindness.

REVIEW

Casting a Genetic Light on the Evolution of Eyes

Russell D. Fernald

Light has been exploited for information by organisms through the evolution of photoreceptors and, ultimately, eyes in animals. Only a handful of eye types exist because the physics of light constrains photodetection. In the past few years, genetic tools have revealed several parallel pathways through which light guides behavior and have provided insights into the convergent evolution of eyes. The gene encoding opsin (the primary phototransduction protein) and some developmental genes had very early origins and were recruited repeatedly during eye evolution. Eye lens proteins arose separately and make up a diverse group, many of which were co-opted from other functions. A major challenge now is understanding how newly discovered pathways for processing light evolved and how they collaborate with eyes to harvest information from light.

Understanding how eyes evolved into what Darwin called an "organ of extreme perfection" (*1*) requires analysis of evolutionary constraints, key selective forces, and possible origins. The evolution of photodetection, giving rise to eyes, offers a kaleidoscopic view of selection acting at both the organ and molecular levels. The repeated exploitation of some regulatory gene sequences in eye development and lens formation raises questions about why certain transcription factors have been regularly recruited to build eyes. The ease with which we can now analyze the evolution of structural gene sequences across species belies the difficulties in tracing the selective forces that shaped regulation of gene expression.

Evolutionary Constraints and Functional Adaptations

Although the variety of eyes in the animal kingdom seems astonishing, physical laws have constrained solutions for collecting and focusing light to just eight types of eye optics (Fig. 1) (*2*). Animal eyes are not simple photon detectors, but organs that produce an image by comparing light from different directions. Biological pinholes, lenses, or mirrors are used to focus an image on photoreceptors (*2*). Light travels in straight lines, and information is carried by wavelength, intensity, and/or polarization, which set limits on eye dimensions and detection systems. Of around 33 animal phyla, about one-third have no specialized organ for detecting light, one-third have light-sensitive organs, and the rest are animals with what we would consider eyes. Image-forming eyes appeared in 6 of the 33 extant metazoan phyla (Cnidaria, Mollusca, Annelida, Onychophora, Arthropoda, and Chordata), and these six contribute about 96% of the known species alive today (*2*).

As earliest evolution occurred in water, which transmits only a limited range of wavelengths, the mechanisms for photon response converged on biochemical solutions that set the course for subsequent evolution (*3*). The evolution of eyes very likely proceeded in stages. First were simple eyespots (early Cambrian period, 570 to 500 million years ago), with a small number of receptors in an open cup of screening pigment. Eyespots would distinguish light from dark but could not represent complex light patterns. Invagination of this eyespot into a pit would add the capacity to detect the direction of incident light. Addition of receptors may then have led to a chambered eye, whereas duplication of an existing pit may have led to a compound eye (*2*). Adding an optical system that could increase light collection and produce an image would later dramatically increase the usefulness of an eye. Whereas primitive eyes can provide information about light intensity and direction, advanced eyes deliver more sophisticated information about wavelength, contrast, and polarization of light.

How many genes might it take to make an eye and how many are expressed exclusively in eye development? Two preliminary answers to the first question from *Drosophila* and mice differ greatly in their estimates. UCLA undergraduates (n = 138) each screened 10 mutant *Drosophila* for eye defects and identified 501 eye-related genes (*4*) or about 3.5% of the *Drosophila* genome. These mutations were distributed among 19 different functional categories (*5*). The largest categories included genes used for signal transduction or regulation of transcription or that were novel. In mice, Williams *et al*. (*6*) reported an expressed sequence tag (EST) library of 15,000 transcripts from ~10,000 genes; ~7500 transcripts were expressed in the retina, regulating both retinal development and function. The hard question is how many genes are used only in development and then play no role in function, and this is completely unknown. Assuming half are associated with development, ~3750 genes are involved, which is 18 times the number in *Drosophila*. However, these estimates are hard to compare for two reasons. First, they are based on quite different techniques. Second, *Drosophila* eyes consist of identical repeated units of photoreceptors, whereas vertebrate retinas are markedly more complex and include photoreceptors and five additional types of processing cells.

Functional constraints have produced nearly identical optical designs in distinctly unrelated animals, most notably fishes and cephalopods. In both lineages, the chambered or camera-like eyes in which an image falls onto a two-dimensional array of photoreceptors are similar in a large number of functional details, despite their great phylogenetic distance (*7*). Invertebrate and vertebrate photoreceptors are distinctly different, most likely arose independently, and are located at the very back of the retina in fish (and all vertebrates) but at the front in cephalopods. Although these eye types are not homologous and the animals carrying them are from distinctly different lineages, there are some homologies among structural and developmental molecules. Both eyes use phylogenetically related forms of opsin as their primary photodetection molecule, and an important regulatory gene, *pax6*, has been found in both vertebrates and some cephalopods, although not in octopus. The use of homologous genes to build nonhomologous structures may lie at the heart of understanding eye evolution and evolutionary processes more generally.

Shared genes may suggest homologous evolutionary paths but may also underlie convergent evolutionary outcomes. For example, the octopus eye arose ~480 million years ago (Mya) and the vertebrate eye 640 to 490 Mya, long after their common ancestor (~750 Mya). Comparing ESTs from octopus eye tissue with those from human eyes revealed ~70% that are commonly expressed, and 97% of these genes are estimated to have existed in the common ancestor of bilaterians (*8*). Overall, about 875 genes have been conserved between humans and octopuses, which may have provided the substrate for the convergent evolution of the camera eye in cephalopods and vertebrates. Among these genes might be a common gene regulatory network recruited at least twice for constructing chambered eyes.

Capturing Photons

The transduction of photons into cellular signals uses seven transmembrane–spanning opsin proteins (30 to 50 kD) that combine with a vitamin A–derived, nonprotein retinal chromophore. Opsins, which control sensitivity to light of different wavelengths, appeared before eyes did (*2*) and evolved into seven [or possibly more (*9*)] distinct families (*10*) (Fig. 2). Opsin was present before deuterostomes split from protostomes

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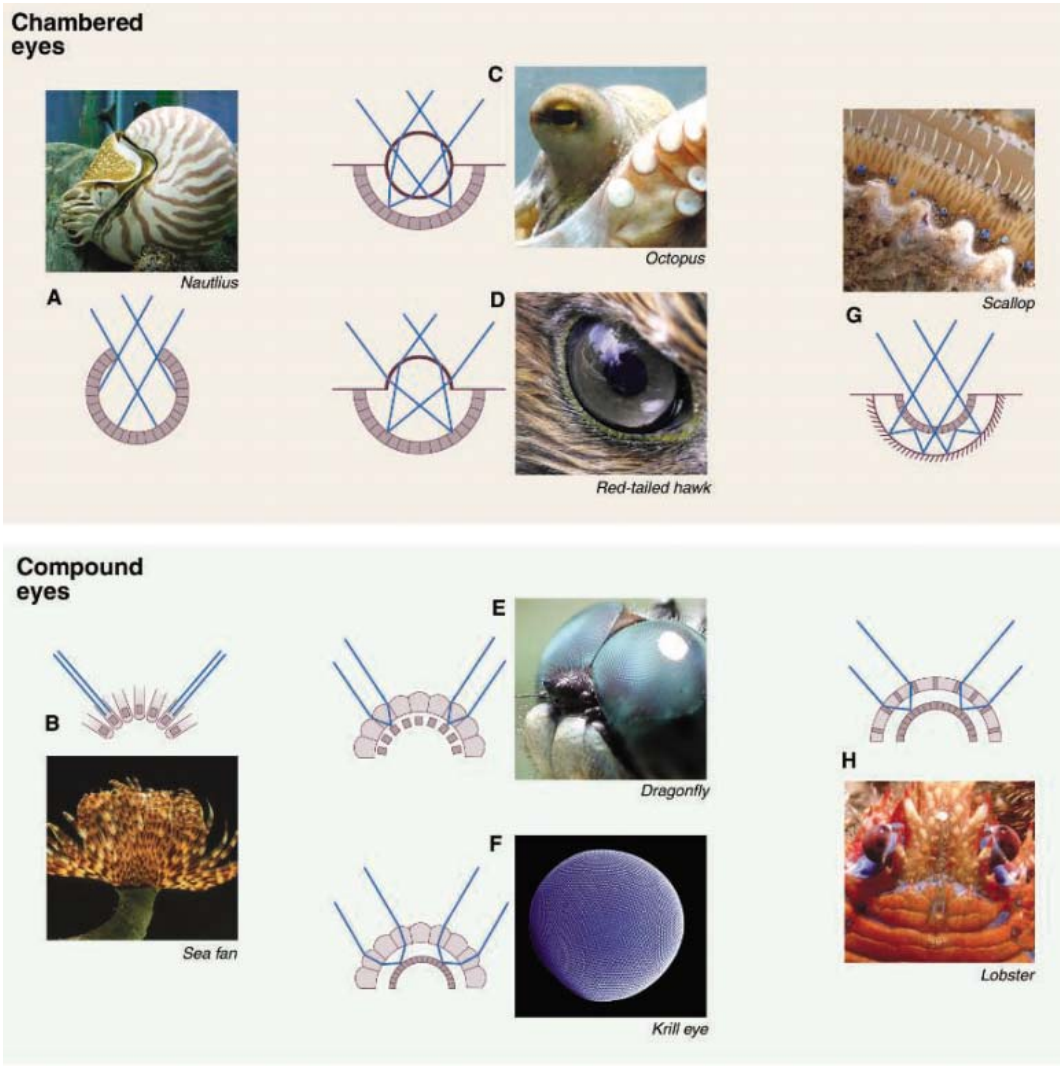

Fig. 1. Eight major types of optics in animal eyes. Both chambered eyes (top) and compound eyes (bottom) form images using shadows (**A** and **B**), refraction (**C** to **F**), or reflection (**G** and **H**). Light rays shown in blue, photoreceptive structures are shaded. The simple pit eye (A) (chambered nautilus) led to the lensed eyes in fish and cephalopods (C) (octopus) and terrestrial animals (D) (red-tailed hawk). Scallop eyes (G) (bay scallop) are chambered but use concave mirror optics to produce an image. The simplest compound eye (B) (sea fan) found in bivalve molluscs led to the apposition compound eye (E) (dragonfly) found in bees, crabs, and fruit flies; the refracting superposition compound eye (F) (Antarctic krill) of moths and krill; and the reflecting superposition eye (H) (lobster) found in decapod shrimps and lobsters. Diagrams modified by permission from (*2*). [Sources: (A) Wikipedia; (B) Robert Pickett/CORBIS; (C) Russell Fernald/Stanford University; (D) Steve Jurvetson/Wikipedia; (E) David L. Green/Wikipedia; (F) Gerd Alberti, Uwe Kils/Wikipedia; (G) Bill Capman/Augsburg College; (H) Lawson Wood/CORBIS]

(*11*). The size of each opsin family is growing rapidly as investigators look at nontraditional organisms and in unexpected places. Multiple new opsin genes, as well as new genes for other phototransduction-specific families [e.g., heterotrimeric guanine nucleotide–binding proteins (G proteins) and nucleotide-gated channels], arose early in vertebrate evolution during extensive chromosome duplications and very likely facilitated retinal specializations (*12*). For example, opsin gene duplication was responsible for the independent evolution of three-color (trichromatic) vision in old and new world primates (*13*). Similarly, opsin gene duplications in Lepidoptera, followed by an increased rate of evolution, produced a diversity of pigments sensitive to visual spectra important for specific species (*14*). Photoreceptor wavelength absorption spectra are exquisitely modulated by a small collection of amino acid side groups adjacent to the chromophore-binding site in the seventh transmembrane domain of opsins, where the effects of natural selection are now most evident (*15*).

An example of how color vision shapes cone opsin evolution is in the visual systems of cichlid fishes in the East African lakes. In one riverine species, ancestral to the lake species, seven cone opsin genes are present as the result of gene duplications. Although only four cone opsins are found in the adult retina and, hence, can contribute to wavelength discrimination by the animal, the rest are expressed at various points during ontogeny. This preservation of opsin genes may offer a substrate for rapid selection of different visual chromatic sensitivities in response to selective pressures (*16*). Another mechanism for modifying the spectral sensitivity is found in bluefin killifish. Animals living in murky swamps have different color sensitivities from those living in clear springs, and the difference is produced through differential expression of cone opsin genes within individual photoreceptors, although how this is regulated is unknown (*17*).

The two best-known photoreceptor types use distinct families of opsins packed in quite different membrane specializations and require different transduction mechanisms (Fig. 3). Vertebrate photoreceptors use members of the ciliary opsin (c-opsin) family incorporated into specialized cilia, whereas invertebrate photoreceptors use members of the rhabdomeric opsins (r-opsin) that are typically formed into rhabdoms. Each receptor type uses different G proteins: transducin in vertebrates and the G_q family in invertebrates. Vertebrate photoreceptors produce hyperpolarizing potentials via a phosphodiesterase cascade; invertebrate photoreceptors are depolarizing and use a phospholipase C cascade. The site of biochemical signal amplification is different between these receptor types, as are the mechanisms for terminating the response. Moreover, opsins in invertebrates are fixed to their membranes (*18*), which allows polarization detection, whereas those in vertebrates are not. It now seems clear that these photoreceptor types arose independently and coexisted in urbilaterians before bilaterians arose (see below).

In using vision to extract information about the environment, all animals exploit the same properties of light: intensity differences to produce contrast and wavelength differences to produce hue. However, no unique solutions exist, and specializations that evolved to process intensity and wavelength differ among species; these differences reflect how similar problems are solved via diverse

mechanisms through natural selection. For example, mammals and bees use long wavelength photoreceptors for intensity and color vision, whereas flies and birds have evolved separate sets of photoreceptors for these two purposes (*19*). The genetic substrates that supported such different evolutionary paths are unknown. Even though blowfly and monkey photoreceptors evolved independently and use different molecular mechanisms, signal processing, and other physiological steps, the information about the world delivered to the nervous system is nearly identical (*20*). These few examples reveal the different routes natural selection has taken during the evolution of eyes in response to the information available in light.

Parallel Universe?

The visual pigments described above are called type 2 opsins to distinguish them from microbial, or type 1, opsins, which are much older and are used for collecting energy and information from photons found in archaea and eukaryotic microbes. Thanks to new techniques for genetic sequencing of samples from fresh and sea water, salt flats, and glacial seas, the number of known type 1 opsins is increasing quickly (currently >800) (*21*). There are striking similarities between opsin types 1 and 2: Both are seven transmembrane–spanning domain proteins, both use an associated retinal moiety to capture light, and, in both, retinal is attached in a Schiff base linkage via a lysine residue in the seventh helix (*21*). However, type 1 opsins differ in physical size and in the distribution of their intramembrane domains, which reflects the differences in their signaling cascades. Type 1 opsins function within the membrane to pump ions or to signal other integral membrane proteins, as opposed to signaling via intracellular G proteins. Finally, the two retinal molecules are photoisomerized quite differently. Researchers were astonished to discover that despite remarkable convergence in molecular details of their function, there is no phylogenetic relationship between them (*21*). So the fundamental mechanism for detecting light using an "opsinlike" protein, associated with retinal, has been discovered and exploited twice independently. Progenitors of the type 1 opsins probably existed in earliest evolution before the divergence of archaea, eubacteria, and eukaryotes, which means that a light-driven ion transport mechanism for deriving energy used in association with opsin 1 preceded the evolution of photosynthesis as a means for using the Sun's energy (*21*).

Lenses

Simple eyes don't have pupils or even lenses, so they can provide only coarse information about the distribution of light in the environment. Lenses allow eyes to collect and concentrate light, which leads to increased sensitivity and allows information contained by that light to be spatially resolved. Advanced eyes collect light through an aperture and focus it with a lens onto photoreceptor cells. As lenses are made from proteins, could the molecular phylogeny of lens proteins instruct us about eye evolution?

Vertebrate lenses are formed from concentric layers of highly elongated fiber cells that differentiate from a peripheral anterior layer of epithelial cells. These contain high concentrations of soluble proteins called crystallins because they maintain transparency. In contrast, the lens proteins of most invertebrate eyes are secreted by specialized cells. A very unusual case is that of a parasite (*Neoheterocotyle rhinobatidis*) in which the lenses are of mitochondrial origin (*22*).

There are three major gene families of crystallins widely expressed in vertebrate lenses that account for most of the protein in aquatic and terrestrial vertebrates: α-crystallins (2 to 3 members), β-crystallins (6+ members), and γ-crystallins (2 to 16 members). It was originally thought that these proteins had uniquely evolved to function as lenses, but some are found expressed in heart, brain, and other tissues of the eye. Recent data reveal that a precursor to βγ-crystallin exists in a urochordate (*Ciona intestinalis*), and functional tests suggest that co-option of ancient regulatory circuits may account for its role in vertebrate lenses (*23*). The remaining vertebrate lens proteins are a diverse, nonconserved group, several of which serve as enzymes elsewhere in the body. Many of these taxon-specific lens proteins have been co-opted from other functions, typically as enzymes, and usually the same gene encodes both the enzyme and lens protein, a process termed "gene sharing" (*24*).

Two taxon-specific lens crystallins, ε (birds and crocodilians) and τ (birds, fish, and reptiles), are active glycolytic enzymes encoded by one gene and demonstrated to be bifunctional (*24*). Such sharing is thought to precede duplication of a structural protein gene, typically followed by specialization of the paralogous genes into different functions. In both duck (*25*) and ostrich (*26*), δ-crystallin genes are bifunctional; they act as metabolic enzymes (argininosuccinate lyase) and lens proteins. In contrast, in chicken, the one (δ1) expressed in the lens has no enzyme activity, and the other (δ2) is enzymatically active (*25*). Similarly, the glycolytic enzyme, lactate dehydrogenase, is a crystalline in crocodilians, elephant shrews, and some birds and is expressed in lenses of various invertebrates. This kind of molecular opportunism is so effective that it has also occurred in both cephalopods (*27*) and *Drosophila* (*28*). One possibility is that because lenses require production of a relatively large amount of protein, genes that have been strongly up-regulated in other tissues might be selected for lens function. Such gene sharing has also been seen to a lesser extent in corneal epithelial tissue, which suggests that certain proteins might be chosen because of a possible role in protecting transparent tissue from ultraviolet radiation (*29*). The common strategy of assembling lenses from diverse proteins seems to be a convergent evolutionary solution that has occurred independently many times in vertebrates. Co-option of taxon-specific ζ-crystallins is thought to have occurred at least three times independently (*30*).

Functionally, the exquisite gradient of refractive index necessary to allow spherical lenses to focus light (*31*) is a convergent solution that has evolved in water-dwelling vertebrates and invertebrates alike. What remains unknown is how genetic programs assemble differing amounts of diverse proteins to preserve the essential functional properties of lenses and whether there is

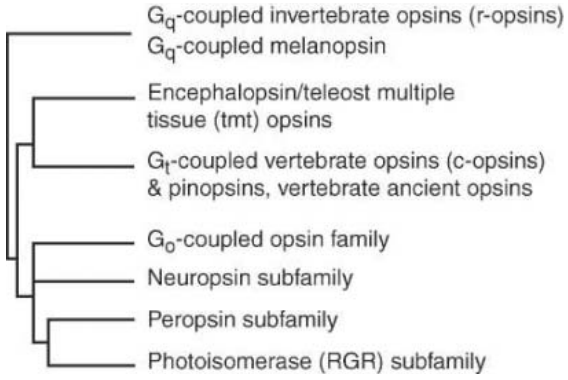

Fig. 2. A simplified schematic molecular phylogenetic tree inferred by the neighbor-joining method showing the seven known opsin subfamilies. Three families transduce light using G protein–coupled mechanisms (G_q, G_t, G_o); the best known are G_q or r-opsins found in invertebrate photoreceptors and G_t or c-opsins found in vertebrate photoreceptors. Encephalopsin and its teleost homolog tmt are found in multiple tissues with unknown function. Pinopsins, closely related to c-opsins, are expressed in the pineal organ of several vertebrates, and vertebrate ancient opsins are expressed in nonphotoreceptor retinal cells, including amacrine and horizontal neurons in teleost fish retinas. Similarly, neuropsins are found in eye, brain, testes, and spinal cord in mouse and human, but little is known about them. Peropsins and the photoisomerase family of opsins bind *all-trans*-retinal, and light isomerizes it to the 11-*cis* form, which suggests a role in photopigment renewal. These are expressed in tissues adjacent to photoreceptors, consistent with this role. Recent data suggest that some cold-blooded vertebrates have an additional opsin type, named parietopsin because it is found only in parietal eye photoreceptors (*9*). [Redrawn with permission from (*11*).]

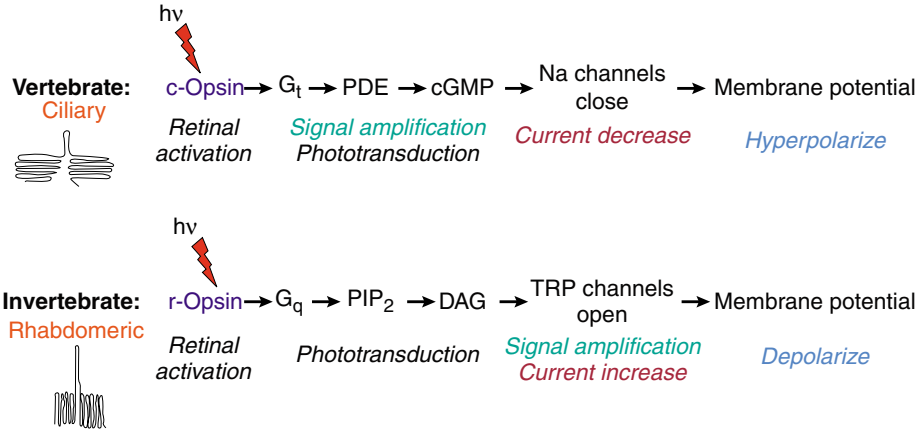

Fig. 3. Schematic illustration showing the key differences between simplified representations of (top) canonical vertebrate ciliary phototransduction and (bottom) invertebrate rhabdomeric phototransduction, where hν represents incident photon energy. The two different opsin types (c-opsin and r-opsin) are contained in distinctly different membrane types, ciliary and rhabdomeric. The opsins are coupled to different families of G proteins that act via different types of transduction cascades. Amplification occurs during phototransduction in ciliary receptors and during channel opening in rhabdomeric receptors. These cascades produce signals of different sign. G_t, transducin; PDE, phosphodiesterase; cGMP, cyclic guanosine monophosphate; G_q, guanine nucleotide–binding protein α15; PIP_2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol.

any rhyme or reason to which specific proteins are used in particular taxa.

Origins of Eyes

Historical views on eye evolution have flip-flopped, alternately favoring one or many origins. Because members of the opsin gene family are needed for phototransduction in all animal eyes, a single origin was first proposed. But subsequent morphological comparisons suggested that eyes evolved 40 or more times independently (*32*); this finding is based on, among other things, the distinct ontogenetic origins of eyes in different species (*33*). For example, the vertebrate retina arises from neural ectoderm and induces head ectoderm to form the lens, whereas cephalopod retinas result from invaginations of lateral head ectoderm, ultimately producing an eye without a cornea. Multiple origins were also supported by an elegant simulation model. Starting from a patch of light-sensitive epithelium, the simulation, under selection for improved visual acuity, produced a focused camera-type eye in less than 4×10^5 generations. For animals with generation times less than a year, this would be less than a half million years (*34*).

The idea that eyes arose multiple times independently was challenged by the discovery that a single developmental gene, *pax6*, can initiate eye construction in diverse species (*35*). However, subsequent work has shown that *pax6* does not act alone and that building an eye requires suites of interacting genes. Discussion about the evolutionary origins of eyes was invigorated by the discovery that homologous genes can trigger construction of paralogous systems for photodetection, just as homologous *hox* genes do for paralogous body parts across phyla (*36*).

Eye development proceeds via morphological transformations of newly generated tissue that are regulated by multiple genes with expression patterns that overlap in time and space. Functions for at least 15 transcription factors and several signaling molecules have been described for human and mouse eye development, many of which are also widely expressed in other tissues. For *Drosophila* photoreceptor arrays, it is now known that seven genes [*eyeless* (*ey*), *twin of eyeless* (*toy*) (both of which are *pax6* homologs), *sine oculus* (*so*), *eyes absent* (*eya*), *dachshund* (*dac*), *eye gone* (*eyg*), and *optix*] collaborate (*37*). These genes, in combination with the Notch and receptor tyrosine kinase pathways and other signaling systems, act via a complex regulatory network (*37*).

Deletion of any one of the seven genes causes radical reduction or complete loss of the *Drosophila* eye. Yet in collaboration with certain signaling molecules, any one of them, except *sine oculus*, can cause ectopic expression of an eye. Like other developmental cascades, a network of genes is required for organogenesis. *Six1*, *Dach*, and *Eya* are important in the formation of the kidney, muscle, and inner ear, as well as eyes, which suggests that this suite of genetically interacting gene products may have been recruited repeatedly during evolution for formation of a variety of structures (*38*).

Appearance of photodetection systems probably happened many (possibly hundreds of) times, until selection produced at least the two independent, main types of photoreceptor types known today—ciliary and rhabdomeric (Fig. 3). The other opsin families likely also have photodetection capacities, mediated by structures still unknown. Although the two main photoreceptor types were thought to be strictly segregated into vertebrates (ciliary) and invertebrates (rhabdomeric), recent studies show that elements of both photoreceptor types probably coexist in most organisms.

An overlooked hint about the existence of multiple photodetection systems came from the discovery of both depolarizing and hyperpolarizing responses to light stimuli from cells located in different layers of a scallop retina (*Pecten irradians*). Depolarizing potentials, characteristic of invertebrate photoreception, arise from the proximal layer, and hyperpolarizing potentials, characteristic of vertebrate photoreception, arise from the distal layer (*39*). In 2004, Arendt and colleagues (*40*) found that the polychete ragworm (*Platynereis dumerilii*) had ciliary photoreceptors in the brain in addition to rhabdomeric photoreceptors in its eyes. The canonical opsins associated with each photoreceptor type were localized only with its type (e.g., vertebrate c-opsin with ciliary receptors in the brain and invertebrate r-opsin with rhabdomeric receptors in the eye). Thus, both main types of "eyes" exist in a worm. Correspondingly, in vertebrates, Berson and colleagues (*41*) had found that a small population of intrinsically photosensitive retinal ganglion cells (the neural output of the retina) use melanopsin, a member of the r-opsin family. Melanopsin in these neurons functions via transduction pathways like those in invertebrates and signals presence or absence of light in parallel to and collaboration with the well-known image-forming visual system (*42*).

Arendt (*43*) proposed that rhabdomeric photoreceptors might be the evolutionary ancestors of vertebrate ganglion cells because of their use of r-opsin and the expression of a constellation of transcription factors including pax6, Math5, Brn3, and BarH. Further, he suggested that other retinal processing neurons, horizontal and amacrine cells, might also share in this rhabdomeric photoreceptor ancestry, but have lost photosensitivity. Taken together, these data show that at least two kinds of photoreception existed in the Urbilateria, before the split into three Bilateria branches at the Cambrian. Moreover, each branch of the family tree still carries versions of both of these photoreceptor types, along with other opsin-dependent photodetection systems yet to be fully described. In the course of evolution, vertebrate vision favored ciliary photodetection for the pathway that delivers images, whereas invertebrates favored rhabdomeric photodetection for their main eyes, although why this might be remains unknown. Along both evolutionary paths, secondary photodetection systems remained to give additional information about light, possibly to instruct

circadian rhythms, phototaxis, or other light-dependent behaviors. But, if vertebrates are an example, these two photodetection systems functioned together, rather than remaining separate. Although the remaining five families of opsins have not been fully characterized, it seems probable that they also respond to light, and organisms use the information they provide.

Genomics and Eye Evolution

For decades, scientists have given considerable attention to the primary imaging system in vertebrates, myopically focused on the function of rod and cone photoreceptors and the visual information they deliver. The discovery that animals have multiple parallel pathways to extract information from light and that these coexist in invertebrates, as well as in the eyes of vertebrates, offers new vistas for discovery in development, function, and evolution of eyes and these other novel systems. Genomics could now be used to identify gene regulatory network kernels, similar to those proposed for body plans, for eyes and their parallel systems. Development in a broader phyletic sample of invertebrate eyes could be instructive in helping identify such developmental networks and also for locating other photosensitive systems. Genetic methods have been used to reveal how photoreceptive ganglion cells interact with conventional photoreceptors functionally in mice, and these techniques could now be extended to identify the functions of the other opsin-based systems. Finally, there are abundant evolutionary questions that might be resolved through genomic approaches. Are the inner retinal neurons actually derived from photosensitive precursors? Are there other convergent optical systems like that of cephalopods and vertebrates with common genetic substrates that could be identified and compared? Is the unusual new opsin identified in the parietal eye (*9*) widespread and will its novel phototransduction system shed light on evolution? Light has been such an important source of information that evolution has exploited it in many ways that remain to be discovered and understood.

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REVIEW

Genomic Evolution of Hox Gene Clusters

Derek Lemons and William McGinnis

The family of Hox genes, which number 4 to 48 per genome depending on the animal, control morphologies on the main body axis of nearly all metazoans. The conventional wisdom is that Hox genes are arranged in chromosomal clusters in colinear order with their expression patterns on the body axis. However, recent evidence has shown that Hox gene clusters are fragmented, reduced, or expanded in many animals—findings that correlate with interesting morphological changes in evolution. Hox gene clusters also contain many noncoding RNAs, such as intergenic regulatory transcripts and evolutionarily conserved microRNAs, some of whose developmental functions have recently been explored.

Hox genes encode a large family of closely related transcription factors with similar DNA binding preferences. They have not been found in sponges, protozoa, or plants but are present in multiple copies in cnidarians and all bilaterian animals. As a distinct branch of the homeobox gene superfamily, Hox genes have been a source of fascination since their discovery because of their powerful functions in diversifying morphology on the head-tail axis of animal embryos. This power is revealed by dramatic duplications of head-tail axial body structures, called homeotic transformations, that can form when one or more of the Hox genes are activated in inappropriate axial positions in developing animals (*1*). The different HOX transcription factors are expressed in distinct, often overlapping, domains on the head-tail body axis of animal embryos (Fig. 1A), and assign different regional fates to these axial domains. As development proceeds, "head" HOX proteins specify the cell arrangements and structures that result in (for example) chewing organs, "thoracic" HOX proteins specify (for example) locomotory organs, and "abdominal" HOX proteins specify (for example) genital and excretory organs. Not surprisingly, extreme homeotic transformations are lethal at early stages of development. Hox genes are also of great interest because there is abundant correlative evidence that changes in Hox expression patterns and protein functions contributed to

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a variety of small and large morphological changes during animal evolution (*2*).

The combination of the aforementioned Hox properties may be the link that relates their functions to the family of MADS box transcription factors that regulate plant developmental patterning. Although structurally unrelated to Hox proteins, the plant MADS box proteins include morphological regulators that have overlapping expression patterns, and similar DNA binding site preferences. Genes encoding plant MADS box proteins can also mutate to provide homeotic transformations of floral structures, and they have been associated with novel expression patterns and functions during plant evolution (*3*).

Variations of Colinear Hox Gene Order

Bilateral animals of the deuterostome (e.g., chordates, echinoderms), ecdysozoan (e.g., arthropods, nematodes), and lophotrochozoan groups (e.g., molluscs, annelid worms, and platyhelminth flatworms) are believed to have evolved from a marine, soft-bodied, wormlike ancestor (Fig. 1B). On the basis of the composition of Hox gene clusters in the diverse animals that evolved from this last common bilateral ancestor, this creature possessed a colinear cluster of at least eight Hox-class genes (*4*, *5*), including one of the *Evx* class (Fig. 2). The original *Evx* homolog (*eve*) was identified in *Drosophila* as being required for normal segment number. However, this segmentation function is not conserved in most animals, where *Evx* genes are expressed in extreme posterior regions of developing embryos and serve axial patterning functions similar to those of other Hox genes (*6*). Hence in Fig. 2, the *Evx* homologs are denoted as *Ev/Hx* to reflect their typical, and presumed ancestral, Hox-like role in tail-region axial patterning.

In some extant animals, the order of Hox genes on the chromosome is roughly colinear with their expression and functional domains on the body axis of embryos (*7*, *8*). The closest to the ideal colinear style of Hox gene arrangement is found on the deuterostome branch in the cephalochordate Amphioxus (*Branchiostoma floridae*) (Fig. 2). The single Amphioxus Hox cluster includes 14 colinear Hox genes closely linked to a pair of *Ev/Hx* genes (*9*). Both short-range, and very long-range, cis-regulatory elements influence multiple Hox promoters and contribute to maintenance of Hox gene clustering and colinearity (*7*, *8*). Recent studies in *Drosophila* have provided evidence that supports direct long-range looping contacts between distant regulatory elements, both enhancers and insulators, with specific Hox promoter regions (*10*, *11*).

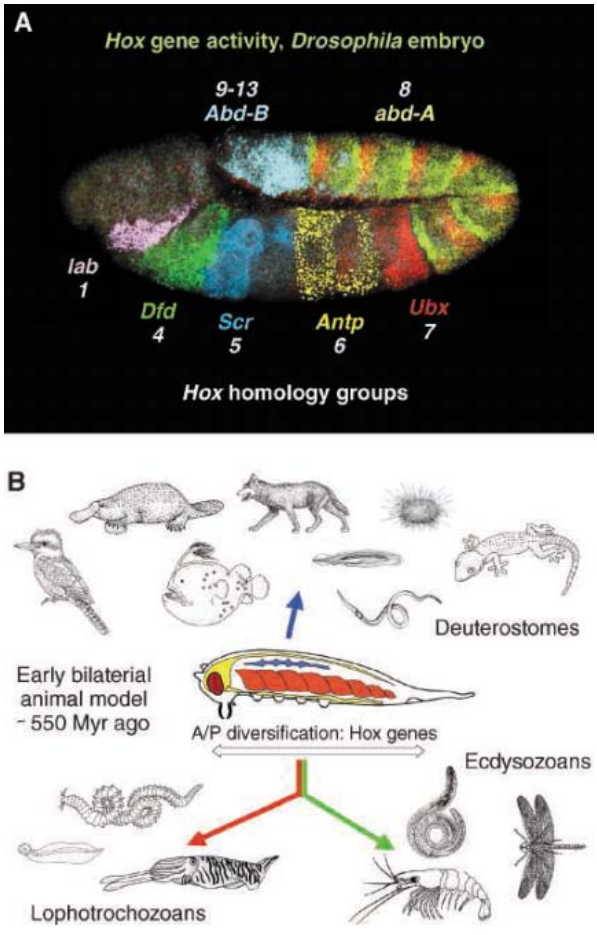

Fig. 1. (**A**) Confocal image of septuple in situ hybridization exhibiting the spatial expression of Hox gene transcripts in a developing *Drosophila* embryo. Stage 11 germband extended embryo (anterior to the left) is stained for *labial* (*lab*), *Deformed* (*Dfd*), *Sex combs reduced* (*Scr*), *Antennapedia* (*Antp*), *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*), *Abdominal-B* (*Abd-B*). Their orthologous relationships to vertebrate Hox homology groups are indicated below each gene. (**B**) Illustration with examples of the diversity of body morphologies produced by the expansion of bilaterial animals. An artist's conception of the hypothetical last common ancestor of all bilateral animals containing muscle tissue (red), a dispersed "central" nervous system (yellow), blood pumping organ (blue), as well as sensory organs and feeding appendages. This ancestor gave rise to all of the extant animals of the three major bilaterian clades (deuterostomes, ecdysozoans, and lophotrochozoans), which was accompanied by the expansion, diversification, and sometimes simplification, of Hox gene clusters.

However, the idea that most animals preserve colinear clusters of Hox genes, and that this is one of their most important properties, is an oversimplification that is falling under the weight of evidence from increasing numbers of animal genome sequences. An extreme divergence from colinearity is found in the urochordate *Oikopleura dioica*, whose genome encodes Hox genes of the anterior and posterior groups, none of which are closely linked (Fig. 2) (*12*). The remaining *Oikopleura* Hox genes are still expressed on the head-tail axis of developing embryos in an order that roughly resembles that of their cephalochordate homologs, although with striking tissue specificities. Another urochordate, *Ciona intestinalis*, shows partial clustering of its Hox gene complement (*13*) but, like *Oikopleura*, is missing many of the Hox genes from the central homology groups.

Mammalian genomes encode four Hox gene clusters (*14*), each of which is missing genes from two or more of the homology groups (Fig. 2), whereas many teleost fishes, having undergone more extensive genomic replications than their mammalian relatives, possess seven partial Hox clusters (*15*). Other deuterostomes, like the sea urchin *Strongylocentrotus purpuratus*, have a single copy of almost the entire complement of Hox homologs, but in a scrambled cluster (*16*).

Among the ecdysozoans, relatively few groups, like nematodes and insects, have had their Hox genomic regions carefully analyzed, although Hox expression patterns are well documented in many arthropods (*17*). In some insect genomes (e.g., the red flour beetle, *Tribolium castaneum*), a colinear Hox cluster is intact. In other insects, like the fruit fly *Drosophila melanogaster*, the Hox cluster is partially fragmented, and many former Hox genes, now called *zen*, *zen2*, *bcd*, *ftz*, and *eve*, have undergone dramatic changes in protein-coding sequence and expression pattern and have adopted novel developmental patterning functions [e.g., (*18*); reviewed in (*19*)]. In another ecdysozoan genome, the nematode *Caenorhabditis elegans*, there has been fairly extensive Hox gene loss and dispersal (*20*), although almost all the remaining genes still play a role in assigning head-tail axial identities during development.

The lophotrochozoan animals have fascinating morphological differences and diverse Hox genes [e.g., (*21*, *22*)], but the best understood Hox gene arrangement at the genomic level is in the platyhelminth flatworm *Schistosoma mansoni* (*23*). This parasitic flatworm has only four Hox genes that are dispersed on two different chromosomes and interspersed with many other unrelated genes. The most ancient extant animals with Hox genes are the cnidarians. Whether they

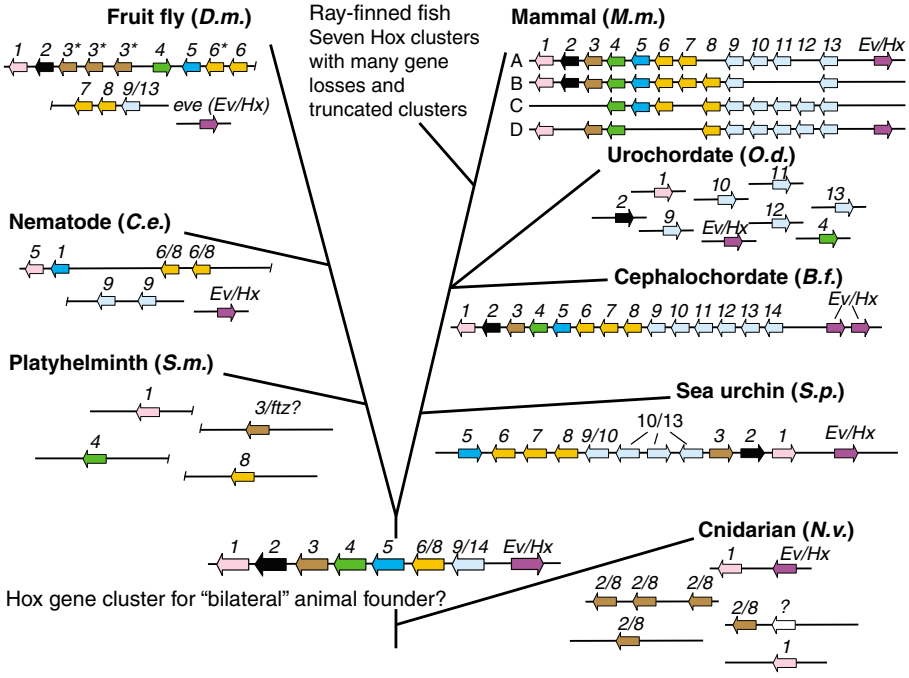

Fig. 2. Cladogram depicting Hox gene chromosomal organization for representative animals. At the base is shown a cnidarian (*Nematostella vectensis*), which has a dispersed genomic organization of Hox genes and lacks posterior Hox paralogs. The left branch displays fragmented Hox clusters for the lophotrochozoan flatworm *Schistosoma mansoni* and the ecdysozoan fruit fly (*Drosophila melanogaster*) and nematode (*Caenorhabditis elegans*). The right (deuterostome) branch portrays the rearranged but coherent Hox cluster of the sea urchin *Strongylocentrotus purpuratus*, the "prototypical" Hox cluster of *Branchiostoma floridae* (a cephalochordate), the dispersed genomic organization of the Hox genes of a urochordate (*Oikopleura dioica*), and the quadruplicated Hox clusters of a mammal (*Mus musculus*), which remain coherent but have experienced losses of multiple paralogs. Similar to the mammals but not shown diagrammatically, the ray-finned fish have multiple duplicate Hox clusters that are mostly coherent and have experienced gene loss, as exemplified by the zebrafish (*Danio rerio*), pufferfish (*Takifugu rubripes*), and medaka (*Oryzias latipes*). At the base of the cladogram is the likely Hox cluster organization of the last common ancestor of bilaterians (*3*). Genes are typically assigned to the Hox class if they encode homeodomain sequences that group with the founder HOX protein sequences from *Drosophila* and vertebrate clusters, and then into Hox homology groups arbitrarily designated 1 through 14. Even having a Hox-like homeobox sequence and mapping in a cluster of Hox genes is not an invariably useful standard for Hox axial patterning function in some animals, because one of the *Drosophila* Hox clusters contains the *ftz* (marked 6* in fly Hox cluster) gene, derived from Hox ancestors, but with novel developmental functions.

have, or their ancestors had, coherent Hox clusters that served oral-aboral ("head-tail") patterning functions is still hotly debated, although multiple cnidarian species possess homologs of bilaterian Hox genes that are expressed in discrete tissue layers on the oral-aboral axis of developing embryos, and at least two cnidarian species have a *Hox1* homolog closely linked to an *Ev/Hx* homolog in a microcluster (*24*, *25*).

Beginning with Lewis (*1*), there has been much speculation that changes in Hox gene number may have contributed to morphological evolution. Such ideas are a variation on the old theory that duplicating genes, particularly developmental control genes, may be an initial step in a process that increases regulatory circuit complexity, leading to increased morphological complexity, whereas elimination of control genes may be an initial step leading to less morphological complexity (*26*). In one case, gene-replacement experiments showed that two mammalian Hox paralogs have nearly identical functions in a variety of tissues (*27*), which may be an example of a Hox duplication and functional divergence at an initial stage.

There is currently no rigorous evidence that connects the loss or gain of specific Hox genes or gene complexes with specific morphological changes in different lineages, but there are a number of intriguing correlations. For example, the axial morphology of Amphioxus appears relatively simple when compared to the diversity of structures on the head-tail axis of large vertebrates such as fish, reptiles, birds, and mammals (Fig. 1B), which have about four times as many Hox genes as cephalochordates. This apparent increase in Hox regulatory complexity may have contributed to the increased morphological complexity of large vertebrates (*28*). In the fish lineage, the teleost (ray-finned) fishes have seven partial Hox clusters, with up to 48 Hox genes in some species, and this is correlated with their great variety in morphology and behavior (*15*). Conversely, the reduced number of Hox genes in some nematodes is correlated with a relatively simple body architecture compared to many of their ecdysozoan relatives (Fig. 1B). In another ecdysozoan, the crustacean *Sacculina carcini* (a barnacle), a striking reduction in abdominal segments is correlated with the loss of the *abdominal-A* Hox gene (*29*). A similar argument can be made for the parasitic flatworm, *S. mansoni*, in which reduced Hox regulatory complexity (Fig. 2) may have contributed to its axial architectural simplicity when compared to other lophotrochozoans such as squids (Fig. 1B), which encode at least nine Hox genes in the sepiolid squid *Euprymna scolopes* (*30*).

On the other hand, adult sea urchins and urochordate tunicates, which exhibit innovative and complex body architectures, have one set of Hox genes, either scrambled or dispersed. Perhaps the novelty of these adult morphologies is dependent on other, equally complex sets of regulatory genes that resemble the Hox genes in their power to diversify morphology but are as yet not well understood.

Noncoding RNAs of the Hox Gene Clusters

Genetic investigations of the *Drosophila* Bithorax Hox gene cluster (BX-C) have revealed a plethora of important developmental regulatory functions, but genetic and molecular studies identified only three lethal Hox complementation groups corresponding to the HOX protein-coding genes *Hox7* (*Ubx*), *Hox8* (*abd-A*), and *Hox9* (*Abd-B*) in Figs. 2 and 3, (*1*, *31*, *32*). Surveys of BX-C transcription have found non–protein-coding transcripts that map to many of the intergenic regions that encode the *bxd*, *iab*, and similar functions (Fig. 3); these regions contain sequences that control levels and patterns of *Ubx*, *abd-A*, and *Abd-B* protein-coding transcripts through a variety of cis and trans mechanisms (*33*–*38*).

Chromatin-remodeling proteins play an integral role in epigenetic regulation and maintenance of proper segment-specific transcriptional states of Hox genes (*39*). Evidence was recently provided for a connection between functions of noncoding Hox cluster transcripts and chromatin-remodeling proteins in Hox regulation (*40*). ASH1 (a member of the Trithorax group of chromatin-remodeling proteins) was found by in vitro binding assays and chromatin immunoprecipitation (ChIP) analysis to bind transcripts in the *bxd* intergenic region near *Ubx* (*40*). These intergenic transcripts are normally generated in the same tissues in which *Ubx* is transcribed, and their association with bound ASH1 was correlated with

specific histone methylation patterns characteristic of derepressed chromatin. Depletion of *bxd* region transcripts by small interfering RNA targeting reduced the ChIP association of ASH1 with the locus and was associated with lower levels of *Ubx* transcription. A similar type of regulation may occur in mammalian Hox clusters, because MLL1 (a vertebrate Trithorax group protein) is found to be associated with extensive regions of the human HOXA complex, including much of the intergenic regions (*41*). These HOXA regions encode many RNAs [as evidenced by EST (expressed sequence tag) clones], some of which, by analogy to *bxd*, might be involved in transcription-dependent chromatin remodeling. However, it remains to be seen if RNA recruitment of ASH1 or other Trithorax group proteins is a curiosity or a more general mode of Hox regulation.

Some of the non–protein-coding transcripts produced in Hox clusters also encode microRNAs (miRNAs). For example, the Hox clusters of many bilaterian animals conserve a sequence for the miRNA miR-10 between their *Hox4* and *Hox5* orthologs (Fig. 3B). In *Drosophila*, miR-10 is predicted to target mRNAs of the neighboring Hox gene *Scr/Hox5* (*42*), although there are not obvious predicted miR-10 targets in the vertebrate *Hox-5* 3′ untranslated region (UTR) sequences.

Arthropod lineages share at least one known miRNA gene, *mir-iab-4*, in the region of the Hox complex containing the abdominal Hox genes (Fig. 3A). Transgene experiments in which a 400–base pair (bp) RNA including the miR-iab-4 hairpin was ectopically expressed in haltere imaginal discs indicate that *Ubx* can be down-regulated by this miRNA in *Drosophila* adult primordia, and patterns of expression of the primary transcript of *iab-4* are complementary to UBX protein expression patterns in embryos (*43*). The relevance of miR-iab-4 to normal developmental patterning awaits the study of mutations that eliminate its function.

At least three of the mammalian Hox clusters encode miRNAs—miR-196a-1, miR-196a-2, and miR-196b—in "abdominal" chromosomal positions similar to those of the *Drosophila iab-4* miRNA, although the miR-196 and miR-iab-4 miRNAs have dissimilar sequences (Fig. 3). A prominent site of expression of miR-196a is in the posterior limb bud, and it has a 1-bp mismatch to a potential target site in the *Hoxb8* 3′ UTR. Analysis of *Hoxb8* transcript cleavage products in the mouse posterior limb bud, and overexpression of a 500-bp RNA containing the miR-196 hairpin in ectopic positions of chick embryos, provide strong evidence that this microRNA cleaves and inactivates *Hoxb8* transcripts (*44*, *45*). All known Hox cluster–encoded miRNAs are expressed in "Hox-like" domains on the head-tail axis of developing embryos (*43*, *46*, *47*), so modification of their expression domains, or gain and loss of Hox mRNA target sites, could vary during evolution to influence HOX protein-dependent morphological functions.

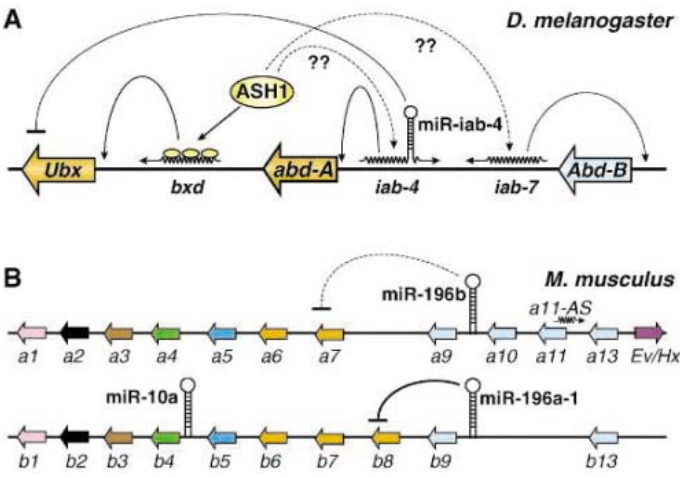

Fig. 3. (**A**) Diagram of the *D. melanogaster* BX-C showing Hox genes (arrows) *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*), and *Abdominal-B* (*Abd-B*), as well as a sampling of noncoding RNAs that derive from the intergenic regions. The intergenic regions *bxd*, *iab-4*, and *iab-7* are transcribed and are known to be involved in proper segment-specific expression of the BX-C Hox genes (interactions with supporting evidence are shown as solid lines). The ability of these regions to affect activation states of the Hox genes is dependent on specific DNA binding sites within these regions (*36*) and on ASH1 binding to noncoding transcripts such as RNAs from the *bxd* region, as well as on regulators possibly binding to other BX-C transcripts (dotted lines). (**B**) Depictions of the mouse *Hoxa* and *Hoxb* clusters. Indicated as hairpins are miRNAs miR-10a, miR-196a-1, and miR-196b along with verified (solid lines) and predicted (dotted lines) interactions with Hox target genes. Also indicated is the position of *Hoxa11* antisense transcripts (*a11-AS*).

Other types of noncoding transcripts, with still undefined genetic functions due to a lack of mutant alleles, have also been discovered in other animal Hox clusters. In a recent survey of Hox gene expression in the centipede *Strigamia maritima*, it was found that transcripts are produced from the 3′ region overlapping the centipede *Ubx* gene, antisense to the direction of *Ubx* transcription (*48*). These transcripts are expressed in the anterior maxillipedal segment, as well as in a subset of neurectodermal cells in the more posterior limb bearing segments in a pattern complementary to that of *Ubx* transcription. The antisense transcript is initiated from sequences downstream of *Ubx* but extends into the 3′ UTR of *Ubx*. Given that the overlapping antisense transcript production precedes *Ubx* protein-coding transcript accumulation, it seems likely that the antisense transcript is involved in inhibition of *Ubx* transcript accumulation (*49*).

A similar example of apparent antisense-mediated regulation was also found in the mouse *Hoxa* cluster in which transcripts are produced from the opposite strand overlapping the *Hoxa11* gene (*50*) (Fig. 3). In situ hybridization detecting the antisense transcripts revealed a pattern of expression in the developing limb buds that is complementary to that of *Hoxa11*, suggesting negative regulation by the antisense transcript. It is also possible that the HOXA11 protein represses the antisense transcript, or that a mutually repressive relationship exists.

Closing Remarks

Genomic analyses have revealed surprising diversity in Hox gene number, organization, and expression patterns in different animals. There are still many animal groups about which little genomic sequence is known, and it remains to be seen how much more variation in Hox gene organization and function will emerge, including the numbers and functions of non–protein-coding RNAs. The property of HOX proteins working as a loosely coordinated system, often with overlapping patterns of expression and function, has apparently fostered their abilities to contribute to morphological change during the evolution of animals. Their colinear arrangement and coordinated regulation in many animals may assist in the maintenance of their overlapping expression patterns. This may have allowed some members of the clusters to subtly and slowly alter their expression patterns and functions to drive groups of cells toward novel structures. But Hox genes still can work as an axial patterning system even when partially dispersed in the genome, and dispersal may foster their rate of functional evolution.

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REVIEW

Gene Regulatory Networks in the Evolution and Development of the Heart

Eric N. Olson

The heart, an ancient organ and the first to form and function during embryogenesis, evolved by the addition of new structures and functions to a primitive pump. Heart development is controlled by an evolutionarily conserved network of transcription factors that connect signaling pathways with genes for muscle growth, patterning, and contractility. During evolution, this ancestral gene network was expanded through gene duplication and co-option of additional networks. Mutations in components of the cardiac gene network cause congenital heart disease, the most common human birth defect. The consequences of such mutations reveal the logic of organogenesis and the evolutionary origins of morphological complexity.

The formation of organs and body parts proceeds by sequential gene regulatory steps that dictate cell fates and organize specialized cell types into complex three-dimensional units of structure and function. Studies of heart development and its genetic underpinnings in simple model organisms and in vertebrates have revealed an evolutionarily conserved gene regulatory network consisting of functional interconnections between myogenic transcription factors, their downstream target genes, and upstream signaling pathways that direct cardiac cell fate, myocyte differentiation, and cardiac morphogenesis (*1*, *2*). Comparative genomic analyses of cardiac developmental control genes and their cis-regulatory elements have also highlighted the conservation of genetic pathways that direct cardiogenesis.

The striking parallels between the transcriptional networks involved in heart development across vast phylogenetic distances support the idea that the evolutionary emergence of hearts with increasing complexity occurred through modification and expansion of an ancestral network of regulatory genes encoding cardiac transcription factors. The expansion of cardiac genetic networks through the duplication of cardiac regulatory genes and the co-option of additional gene networks probably allowed for the addition of new accessory structures, such as chambers, valves, and a conduction system, to a primitive vessel-like heart analogous to that of invertebrates and vertebrate embryos (*3*). The modular addition of innovations to primitive structures, although speculative, has also been proposed as a mechanism for the genesis of other vertebrate organs and body structures (*4*). Insights into the genetic circuits that drive the evolution and development of the heart shed light on general principles of organogenesis and evolutionary origins of morphological complexity, as well as the molecular basis of cardiovascular disease in humans.

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Evolutionary Advancements of the Heart

The most fundamental functional units of all hearts are cardiac muscle cells, which express an array of contractile proteins, such as muscle actin, myosin, troponin, and tropomyosin. The appearance of muscle cells preceded the divergence of Cnideria (hydra, jellyfish, and corals) and Ctenophora (comb jellies) from the Bilateria, from which mammals descended (~700 million years ago) (Fig. 1) (*5*). Primordial muscle cells probably resembled the epithelio-muscle cells of Cnideria and amphioxus, which is thought to be the closest living approximation of the invertebrate ancestor of vertebrates (*6*–*8*). These cells probably existed in a primitive gastric pocket where they participated in fluid movement during feeding. Muscle cells in bilaterians are derived from mesoderm, which is believed to have arisen from the gastrodermis of a diploblastic ancestor. The diversification of muscle cells gave rise to skeletal, cardiac, and smooth muscle cells, and further specialization of cardiac muscle cells ultimately yielded atrial and ventricular myocytes, as well as the cells of the mammalian cardiac conduction system.

The first heart-like organ is believed to have appeared over 500 million years ago in an ancestral bilaterian (*6*, *9*–*11*). It probably resembled the simple tubular vessel-like organs of tunicates and amphioxus, which contain a

myoepithelial cell layer, lacking defined chambers or valves (Fig. 2). The heart of *Drosophila*, referred to as the dorsal vessel, also functions as a linear peristaltic pump but, in contrast to the hearts of tunicates and amphioxus, it ends in a closed cardiac compartment and contains a cardioaortic valve that separates a posterior lumen and an anterior aorta-like structure (Fig. 3) (*1*, *2*). Nematodes do not possess a heart per se, but their pharynx contracts like a heart, and the muscle cells that line its walls exhibit electrical activity similar to that of mammalian cardiomyocytes (*12*).

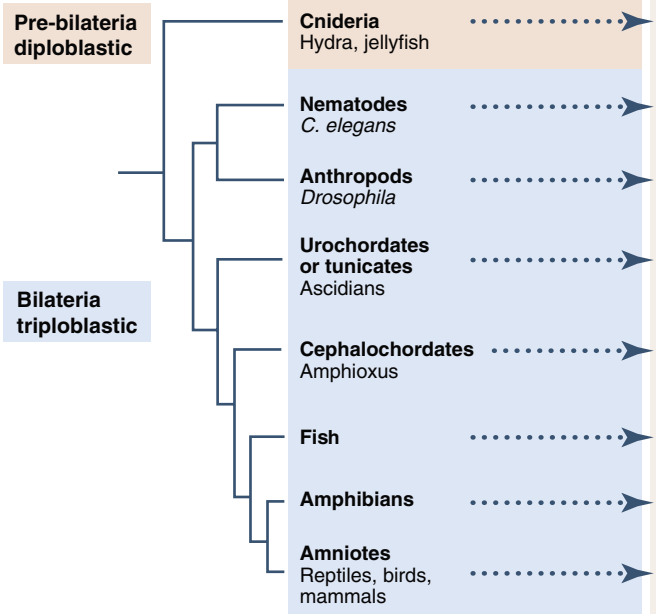
During evolution, the heart evolved from a single-layered tube with peristaltic contractility to a more efficient and powerful pump with thick muscular chambers dedicated to receiving (atrial) and pumping (ventricular) blood, displaying synchronous contractions and seamless connections to a closed vascular system (*10*, *11*). The transition from an aquatic to a terrestrial environment required several additional adaptations of the heart to separate oxygenated and deoxygenated blood (Fig. 2). The hearts of fish contain a single atrial chamber connected directly to a ventricle. Amphibians have two atria separated by a septum and a single ventricle. Terrestrial vertebrates have divided hearts in which septae separate the oxygenated and deoxygenated blood within the pulmonary and systemic circulations. Efficient unidirectional blood flow into and out of the heart was ensured by the appearance of valves. The conversion of a primitive heart tube to a multichambered heart that drives blood at high force through synchronous contractions also required a conduction system. Other advancements of the vertebrate heart include neural crest cells, which contribute to portions of the outflow tract and septum; trabeculae, which enhance oxygenation; the endothelium, which provides growth factor signals and precursor cells for formation of the cardiac valves; and the epicardium, which provides precursors for the coronary vasculature.

An Ancestral Genetic Network for Heart Development

Heart development is governed by a core set of evolutionarily conserved transcription factors (NK2, MEF2, GATA, Tbx, and Hand) that controls cardiac cell fates, the expression of genes encoding contractile proteins, and the morphogenesis of cardiac structures (Fig. 1). These transcription factors also regulate each other's expression, serving to stabilize and reinforce the cardiac gene program (*1*, *13*–*15*). Dozens of other transcription factors contribute to cardiogenesis, in many cases by serving as accessory factors for these core regulators.

The MADS-box protein MEF2, which is conserved throughout the metazoans and exists even in yeast, is the most ancient myogenic transcription factor and presumably became irreversibly committed to the expression of muscle genes in an ancestral organism (*16*). As muscle cells diversified, MEF2 became a central component of muscle gene regulatory networks and is the only myogenic transcription factor known to be associated with the differentiation of all muscle cell types. In cardiac muscle cells, MEF2 cooperates with the core cardiac transcription factors to regulate contractile protein gene expression, whereas in skeletal muscle MEF2 cooperates with the MyoD family of bHLH transcription factors (*16*). Thus, MEF2 appears to have coopted different transcriptional partners to regulate different muscle gene programs via specific combinations of cis-regulatory sequences.

Within cardiac muscle lineages, *Mef2* fell under the control of NK2-type homeodomain proteins, which became dedicated to cardiac muscle and associated endodermal structures (*1*, *13*, *14*, *17*). *Mef2* and an *NK2* homeobox gene closely related to those involved in the cardiac development of bilaterians are expressed in myoepithelial cells within the gastrodermis of Cniderians, which do not contain a heart (Fig. 1), suggesting that these genes were already associated with the muscle gene program in the common ancestor of these organisms (*8*). It has been postulated that a layer of muscle cells developing under the direction of an NK2-class gene within the endoderm of an ancestral organism, which may have been Cniderian-like, evolved into pulsatory muscular vessels of an early bilaterian (*17*).



Cardiac Structures	Cardiac Expression of Network Genes				
	NK2	MEF2	GATA	Tbx	Hand
Striated muscle cells associated with gastrodermis	1	1	?	1	0
Contractile pharynx	1	1	2	1	0
Tubular heart Open circulatory system	1	1	2	3	1
Tubular heart, striated Open circulatory system Bidirectional pumping	1	1	2	≥3	1
Tubular heart, non-striated Closed circulatory system Unidirectional pumping	1	1	2	?	1
Single atrium and ventricle	≥3	4	3	≥4	1
Two atria and one ventricle Pulmonary circulation	≥3	4	3	≥5	1
Four-chambered heart	≥2	4	3	≥7	2

Fig. 1. Evolution of the heart and the core cardiac transcription factors. The structures of the hearts of representative animals and their evolutionary relationships are shown. The numbers of cardiac regulatory genes, which are known to be expressed in the cardiac structures of each organism, are shown.

The conservation of the core cardiac transcription factors and their cardiac expression in all modern-day organisms with hearts suggest that they became coupled to the expression of muscle genes involved in contractility and pump formation in an ancestral protochordate, and such regulatory interconnections were maintained and elaborated on during the evolution of more complex cardiac structures. Gene duplications during evolution increased the number of genes encoding these core cardiac transcription factors (Fig. 1). Such duplications, coupled with the modification of cis-regulatory elements, generated new patterns of gene expression; and variation in protein-coding regions conferred specialized activities, allowing the acquisition or modification of cardiac structures and functions. Consistent with the idea that new gene family members became more specialized and/or that functional redundancies masked their shared or subtle functions, mutations in cardiac regulatory genes in *Drosophila*, in which the cardiac regulatory network is relatively simple, often result in dramatic abnormalities in cardiac development, whereas mutations of individual paralogs of these genes in vertebrates frequently affect specific structures of the heart (such as ventricles or valves) that do not exist in the hearts of insects or more primitive organisms (*1*).

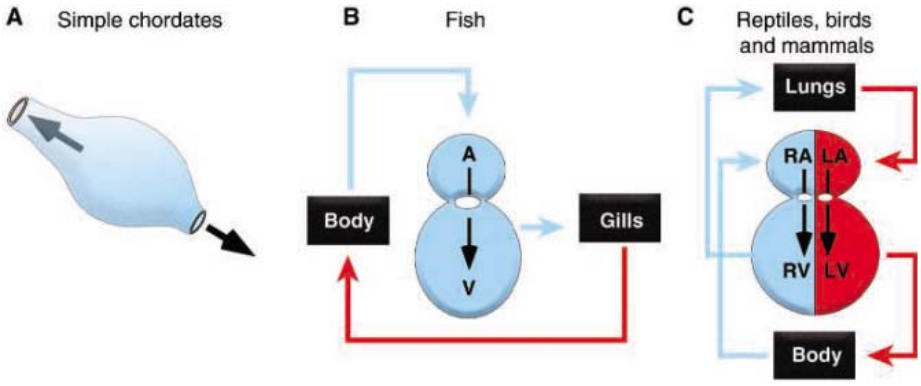


Fig. 2. Simplified structures of different types of hearts, showing schematic diagrams of hearts and directions of blood flow. (**A**) Simple chordates have tubular hearts, some of which pump bidirectionally. The hearts of ancestral bilaterians probably had a similar structure. Fish hearts (**B**) have a single atrium and ventricle, whereas the hearts of reptiles, birds, and mammals (**C**) have two atrial and two ventricular chambers. Oxygenated blood is shown in red and deoxygenated blood in blue.

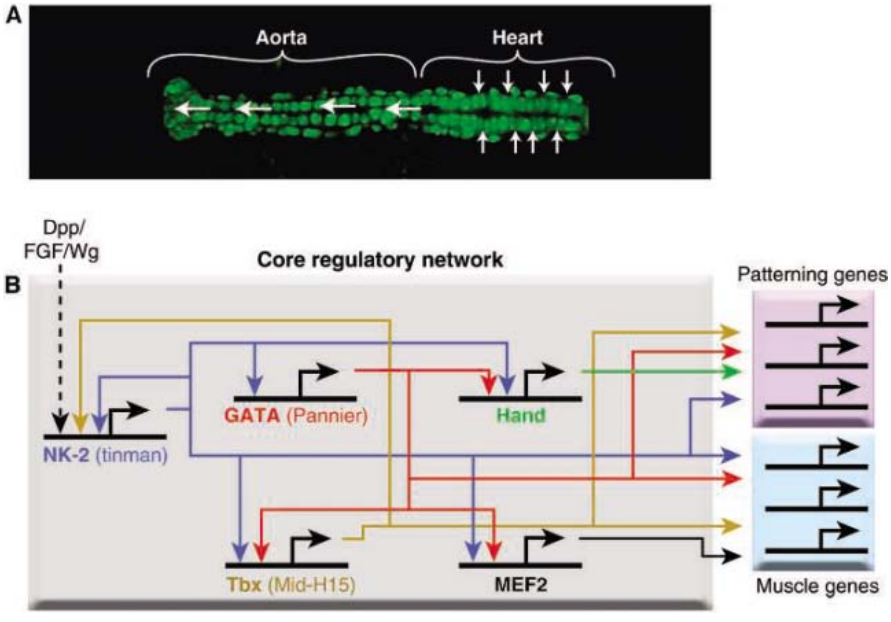


Fig. 3. The heart of *Drosophila*. (**A**) The heart of a *Drosophila* embryo visualized by the expression of green fluorescent protein under control of the cardiac enhancer of the *Hand* gene. Hemolymph enters the heart at the posterior end of the embryo through ostia and is pumped anteriorly. (**B**) A simplified diagram of the core transcriptional network of *Drosophila* heart development.

Gene Networks in *Drosophila* Heart Development

Drosophila has provided a powerful model for delineating the architecture of the cardiac regulatory network, due to the relative lack of functional redundancies in that network (Fig. 3). Formation of the dorsal vessel requires signaling by Decapentaplegic (Dpp), a member of the transforming growth factor–β superfamily; fibroblast growth factor (FGF); and wingless (Wg), which belongs to the Wnt superfamily (*1*). The *Drosophila* NK2 homeobox gene, *tinman*, is essential for the specification of cardiac cell fates (*18*, *19*) and serves as a target of inductive signals for cardiogenesis (Fig. 3). Among the target genes of *tinman* is the *Mef2* gene, which is required for the differentiation of all types of muscles. Loss of function of the single *Mef2* gene in *Drosophila* abolishes the expression of contractile protein genes in cardiac, skeletal, and visceral muscle cells but does not affect muscle cell identity, demonstrating the dedication of this factor to muscle differentiation.

Mutation of the GATA gene, *pannier*, in *Drosophila* results in an absence of cardioblasts and a decrease in the number of pericardial cells (*20*). *tinman* expression is lost in *pannier* mutants, and ectopic expression of *pannier* results in the production of supernumerary cardioblasts. Multiple T-box genes function together with *tinman* and *pannier* to control cardiac fate, differentiation, and patterning of the dorsal vessel (*21*, *22*). The *Drosophila* genome encodes a single member of the Hand family of bHLH transcription factors, which is directly regulated by Tinman and Pannier and is required for normal development of the dorsal vessel (*23*, *24*). Autoregulatory and cross-regulatory interactions of *tinman*, *Mef2*, *pannier*, *T-box*, and *Hand* genes maintain the cardiac phenotype once the network has been activated by upstream inductive signals.

Adding New Units of Structure and Function to the Vertebrate Heart

In vertebrate embryos, cardiac precursor cells are specified in the lateral mesoderm by signals from adjacent tissues, many of which are conserved in organisms ranging from insects to mammals (*1*, *2*). Cardiac progenitors from the primary heart field converge at the ventral midline to form a linear heart tube that resembles, both structurally and functionally, the primitive heart thought to exist in ancestral chordates. Development of the heart tube into the mature multichambered heart requires multiple steps that depend on genetic programs unique to vertebrates and/or amniotes. The heart tube gives rise to the left ventricle of the four-chambered heart, which is believed to represent

the ancestral chordate cardiac compartment (*13*). The right ventricular chamber and outflow tract, later evolutionary advancements, are formed primarily from an adjacent population of precursors, referred to as the secondary or anterior heart field (*25*–*27*). Although there is debate as to whether this supplemental cell population represents a separate heart field versus an expanded region of the primary heart field, it is reasonable to conclude that the evolutionary addition of the right ventricle, as a new unit of cardiac structure and function, occurred through the recruitment of a novel population of precursor cells to a preexisting organ, rather than by simply expanding a common field of precursor cells. The hearts of fish and amphibians lack a right ventricle but contain a rudimentary outflow tract, a structure derived from the secondary heart field in mammals. It is currently unclear whether the outflow tract in these organisms is derived from the beginnings of a secondary heart field. It is also conceivable that the secondary heart field was first invented not to provide a new ventricle but simply to increase the mass of the original single ventricle, in which case it could have appeared in evolution before the second ventricle was invented.

At the posterior end of the heart tube, signaling by retinoic acid, a vertebrate invention, establishes atrial identity in cells that would otherwise adopt a ventricular fate (*11*), thereby conveying positional information along the anterior-posterior axis to chamber-specific genes. In this case, a new signaling pathway was coupled to the ancestral cardiac regulatory network to generate a new structure. Portions of the heart tube that do not become chamber myocardium give rise to the cardiac conduction system through genetic pathways that are also unique to vertebrates.

Expansion of the Ancestral Cardiac Gene Network in Vertebrates

Expansion of the number of ancestral cardiac regulatory genes (Fig. 1) and modification of the timing and pattern of their expression, as well as their regulatory interactions with each other and with other developmental control genes, was undoubtedly a major driving force for building cardiac complexity during evolution. Cardiac genes are typically controlled by combinations of cis-regulatory elements that operate in distinct regions of the heart (*28*). It is not unusual, for example, for separate enhancers to direct transcription of a gene in the right versus the left ventricle or even within subdomains of these structures that are otherwise indistinguishable. This diversity of regulatory elements has the potential to generate highly specialized groups of cells with distinctive gene expression profiles, allowing for the evolutionary modification of specific cardiac structures without affecting the entire organ. Such modularity in gene regulation is also revealed by the restriction of many cardiac defects to specific anatomical regions of the heart (*14*).

The homeobox gene *Nkx2-5*, a homolog of Drosophila *tinman*, is expressed in cardiac progenitor cells and the associated endoderm of all chordates, as well as urochordates and cephalochordates, and serves as a target of inductive signals that initiate cardiogenesis (*7*, *17*, *28*–*30*). Forced expression of *Nkx2-5* in zebrafish or frog embryos expands the heart field and promotes cardiac gene expression (*31*, *32*), whereas a dominant-negative Nkx2-5 mutant protein can block cardiogenesis in frog embryos; combined expression of Nkx2-5 and Nkx2-3 dominant-negative mutants results in a more severe phenotype than do mutants of either protein alone (*33*). The latter results have been interpreted as an indication of redundancy between these factors in the developing heart. However, overexpression of dominant-negative mutant proteins may also disrupt the activities of multiple transcription factors, particularly in the setting of heart development, in which transcription factors interact combinatorially, and may therefore result in more severe phenotypes than gene deletions. In mice lacking *Nkx2-5*, the initial events of heart formation occur normally, but embryos die from abnormal morphogenesis of the heart tube and failure in left ventricular development (*34*); defects that can be interpreted as a selective loss of derivatives of the primary heart field. The disparity between the essential early role of *tinman* in specification of the cardiac lineage in *Drosophila* and the relatively late cardiac defects in *Nkx2-5* mutant mice could be explained if other NK2 homeodomain proteins, or other cardiac transcription factors, substituted for an early function of Nkx2-5.

Of the four vertebrate *Mef2* genes, *Mef2c* is required for activation of a subset of cardiac contractile protein genes, as well as for the development of cardiac structures derived from the secondary heart field, which are unique to amniotes (*35*). Thus, it appears that during evolution, this ancient myogenic regulator acquired new functions in regulating the formation of cardiac structures that occur only in more advanced hearts. Additional functions of vertebrate *Mef2* genes are likely to be masked by redundancies.

Members of the GATA family of zinc-finger transcription factors directly regulate numerous cardiac contractile protein genes, as well as upstream regulatory genes such as *Nkx2-5*, *Mef2*, and *Hand* (*20*). Of the six GATA genes in vertebrates, three (*Gata4*, *-5* and *-6*) are expressed in the heart and have been implicated in heart development through loss-of-function mutations. Forced expression of cardiac GATA factors in *Xenopus* and zebrafish embryos induces premature activation of cardiac gene expression (*36*, *37*).

At least seven Tbx genes display overlapping expression in the primary and secondary cardiac lineages and other cardiac structures of amniotes (*38*). Mice lacking Tbx5 display defects in the posterior region of the heart tube from which the atria are derived. Expression of a dominant-negative Tbx5 mutant in *Xenopus* embryos prevents formation of the primitive heart. *Tbx5* and *Tbx20* have also been implicated in formation of the cardiac conduction system and ventricular chambers, respectively. Tbx2 and Tbx3 function as repressors of chamber myocardium and are associated with the development of the conduction system (*10*).

The correlation between gene duplication and cardiac complexity is especially intriguing with respect to the *Hand* genes, which regulate ventricular growth (*13*, *14*). Amphibians and fish, which contain only a single ventricle, express only one *Hand* gene, and zebrafish mutants lacking the *Hand* gene fail to form the ventricular chamber (*39*). In mice, *Hand1* and *Hand2* are preferentially expressed in derivatives of the primary and secondary heart fields, respectively (*13*). Mice lacking *Hand2* do not form a right ventricle, probably reflecting ablation of the secondary heart field (*40*), and *Hand1* mutant embryonic stem cells are unable to contribute to the outer curvature of the heart that gives rise to the left ventricle (*41*). Deletion of *Hand2* and *Nkx2-5*, which regulates *Hand1* expression in the primary heart field, eliminates both ventricular chambers, leaving only an atrial remnant (*13*, *14*). Thus, evolutionary duplication of the ventricular chambers correlates with duplication of the *Hand* genes.

Building Cardiac Complexity by Co-opting New Genetic Networks

The co-option of different upstream inputs by the core cardiac gene network appears to have played an important role in the evolution of the heart. In the tunicate Ciona, the single *Mesp* gene, encoding a bHLH transcription factor, is expressed in cardiac progenitors and is required for the expression of *NK2* and *Hand* genes (*29*, *42*). Similarly, the two *Mesp* paralogs in the mouse (*Mesp1* and *-2*) are redundantly required for formation of the cardiac mesoderm. In contrast, *Mesp* is not expressed in precursors of the *Drosophila* heart, suggesting that *Mesp* and its regulatory network were recruited to act upstream of the ancestral cardiac gene network during chordate evolution, or that the connection of *Mesp* to the cardiac network was lost during the evolution of insects.

Varying the upstream inputs to the cardiac regulatory network also provides an explanation for the development of the right ventricular chamber from the secondary heart field. Cardiac muscle cells in both the right and left ventricles rely on the same set of transcription factors for activation of the gene program for cardiomyocyte differentiation and the expression of con-

tractile protein genes, but the upstream inputs into this regulatory network differ in cells derived from the primary and secondary heart fields (Fig. 4A). The evolutionary addition of the secondary heart field required a signaling mechanism to activate the core cardiac transcriptional network. The Isl1 transcription factor, which is expressed specifically in the secondary heart field (*26*), directly activates the *Mef2c* gene in this population of cardiac precursor cells (Fig. 4B) (*43*). In this case, Isl1 was connected to the cardiac regulatory network, possibly through the acquisition of Isl1-dependent enhancer modules by *Mef2c* and perhaps other core cardiac regulatory genes. Because Isl1 is not cardiac-specific, its initial activation and its actions on downstream targets require combinatorial mechanisms with other factors or epigenetic influences. GATA factors and Nkx2-5, which are expressed in both heart fields and are required for *Mef2c* expression in the secondary heart field, may serve this role. The forkhead transcription factor Foxh1 also activates *Mef2c* transcription in the secondary heart field through a separate enhancer and appears to act downstream of Isl1 (*44*). Mutations in *Isl1*, *Mef2c*, or *Foxh1* all result in severe cardiac defects that appear to reflect ablation of the secondary heart field and its descendent structures (*26*, *35*, *44*), demonstrating the interdependence of these cardiogenic regulators.

These findings suggest that the evolution of the four-chambered heart involved the acquisition of a new set of regulatory inputs into the ancestral cardiac transcription factor genes. Because genes within the core cardiac network cross-regulate and autoregulate their expression, activation of one or a few of the genes in the network may ultimately activate them all, as well as common sets of downstream genes. The regulation of the core set of myogenic transcription factors by different upstream signals in different cardiac muscle precursor populations is reminiscent of the strategy for skeletal muscle development in which members of the MyoD family are regulated by different upstream signals and transcriptional inputs in different skeletal muscle lineages, thereby contributing to muscle diversity and specialization (*45*).

Downstream Targets of Cardiac Transcription Factors

Relatively little is known about the downstream target genes of the core cardiac transcription factors that drive cardiac growth and morphogenesis. How cardiac looping occurs and how the ventricular chambers adopt their specific shapes and positions are important unanswered questions. Morphogenesis and growth of the heart are intimately connected to cardiac function, but the mechanistic basis of this link is also vague, as are the mechanisms whereby the heart (or other organs) coordinates its size with that of the body. Molding of the cardiac chambers depends on myocyte differentiation and contractile activity as well as blood flow, fluid dynamics, and oxygenation. How these intrinsic and extrinsic influences govern the growth and development of the heart is unclear.

In addition to the structural and regulatory genes that control cardiac development and contractility, several evolutionarily conserved microRNAs (miRNAs), which function as negative regulators of target RNAs, are expressed specifically in the developing heart. One such miRNA, *Mir1*, negatively regulates cardiac growth during mouse development by inhibiting translation of Hand2 (*46*). In *Drosophila*, *Mir1* is required for proper patterning of the dorsal vessel, but the *Drosophila Hand* mRNA is not a target of *Mir1*, suggesting that other evolutionarily conserved

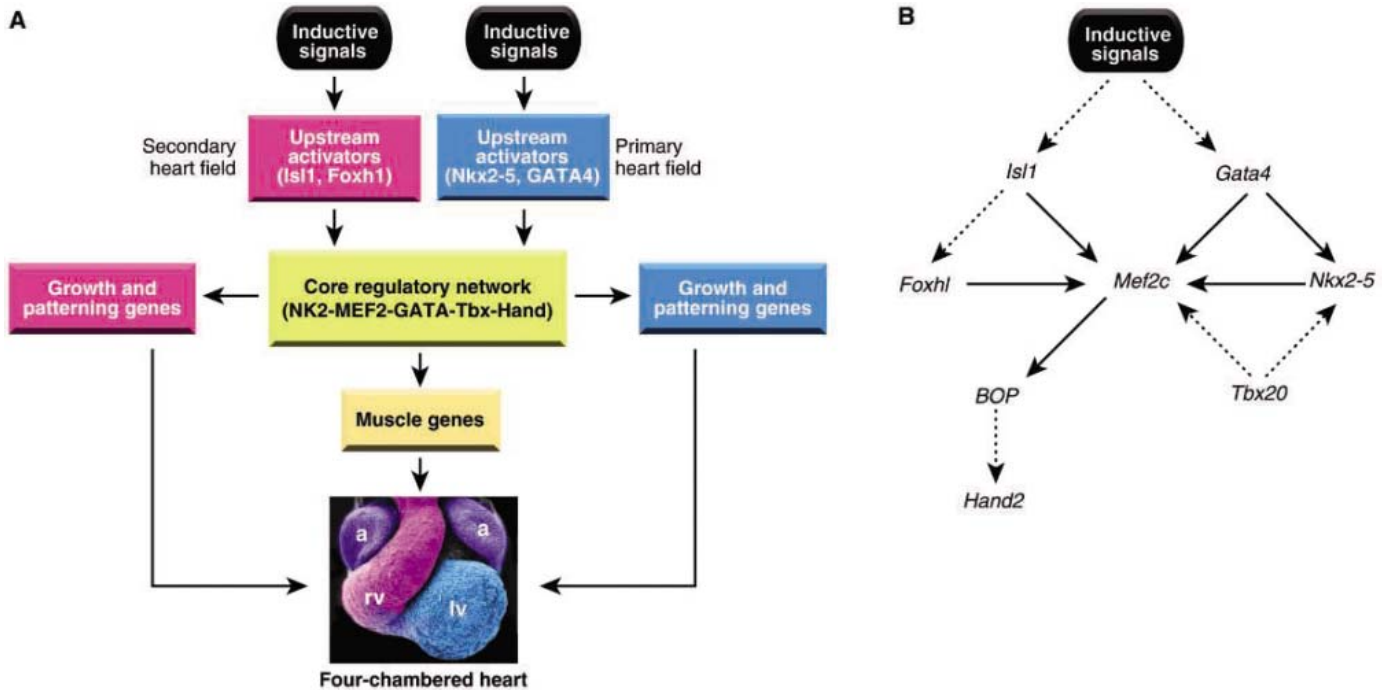


Fig. 4. Schematic of transcriptional networks involved in mammalian heart development. (**A**) Inductive signals activate a set of upstream regulatory genes, encoding transcription factors, in the primary and secondary heart fields. The products of these genes activate the genes in the core cardiac network (NK2-MEF2-GATA-Tbx-Hand). Some components of the network, such as Nkx2-5, are also activated in the primary heart field in response to inductive signals. The core network genes cross- and autoregulate their expression and serve as the central regulatory network for the activation of muscle-specific genes and genes that control the growth and patterning of derivatives of the primary and secondary heart fields. The primary heart field gives rise to the left ventricle (lv) and portions of the atria (a), whereas the secondary heart field gives rise to the right ventricle (rv), portions of the atria, and the outflow tract. A scanning electron micrograph of a mouse heart at embryonic day 14.5 is shown at the bottom. Derivatives of primary and secondary heart fields are shown in blue and pink, respectively. The atria, which are derived from the primary and secondary heart fields, are shown in purple. (**B**) Regulatory interactions among cardiac transcription factors in the secondary heart field. Solid lines indicate direct transcriptional connections, and dotted lines indicate connections not yet shown to be direct. [Adapted from (*9*)]

targets may exist or that *Mir1* acts differently in insect and mammalian heart development (*47*). Understanding the roles of miRNAs in heart development and disease represents a rich area for future investigation.

Insights into Human Heart Disease

Heterozygous mutations in cardiac regulatory genes frequently cause congenital heart disease in humans, illustrating the exquisite sensitivity of cardiac structure and function to genetic perturbation. Mutations in Nkx2-5 cause a spectrum of congenital heart defects (*48*), including cardiac conduction abnormalities, ventricular-septal defects (VSDs), and atrial-septal defects (ASDs). Mutations in Tbx5 are responsible for Holt-Oram syndrome (*49*), an autosomal dominant disorder associated with structural and functional cardiac defects, and deletion of Tbx1 results in malformations of the cardiac outflow tract and VSDs due to failure in the migration of neural crest cells to the heart (*50*). Mutations in GATA4, some of which disrupt its interaction with Tbx5, cause ASDs and VSDs (*51*). The realization that heart defects in humans often result from haploinsufficiency of cardiac transcription factors suggests that strategies to enhance the activity of such developmental regulators, even subtly, may provide therapeutic benefit.

The discovery of cardiac regulatory gene networks has allowed for genetic testing for cardiac disease genes. However, congenital heart disease in humans commonly displays variable penetrance and expressivity, pointing to the influence of modifier genes and environmental influences on cardiac phenotypes. Understanding the molecular basis of such variability is an important challenge for the future.

Warm-blooded animals are unable to effectively repair the injured myocardium, whereas amphibians, fish, mollusks, and arthropods can replace lost cardiac myocytes through regeneration, suggesting that the ability to undergo cardiac regeneration may represent a primordial metazoan attribute that was lost. There is evidence for a contribution of stem cells to repair of the mammalian myocardium, but this endogenous mechanism is inadequate to restore function to the failing heart. In addition to their roles in heart formation, many developmental regulators, such as MEF2, GATA4, and Nkx2-5, are redeployed after injury to the adult heart and ensuing changes in cardiac contractility and function (*52*). There is great interest in therapeutically manipulating the activities of these transcription factors in the adult heart to promote cardiac repair, including the genesis of specialized cardiac cell types from stem cells for cellular replacement.

Further analysis of the genetic networks that govern heart development through the combined use of genomics, genetics, and model organisms promises to yield insights, not only into general principles of organogenesis, but also to facilitate therapies for congenital and acquired heart disease.

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Elevated Eocene Atmospheric CO_2 and Its Subsequent Decline

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Quantification of the atmospheric concentration of carbon dioxide ($[CO_2]_{atm}$) during warm periods of Earth's history is important for predicting global warming, because over the next 100 years burning of fossil fuels may produce $[CO_2]_{atm}$ approaching 1000 parts per million by volume (ppm) (*1*). One such warm period, the early Eocene [~56 to 49 million years ago (Ma)], had the highest prolonged global temperatures of the Cenozoic, peaking ~52 to 50 Ma during the early Eocene climatic optimum (EECO) (*2*). However, the relation between atmospheric CO_2 concentrations and greenhouse climates of the early Eocene is uncertain because proxy measurements from paleosols (*3*, *4*), marine boron isotopes (*5*), and leaf stomatal indices (*4*) give estimated $[CO_2]_{atm}$ concentrations between 100 and 3500 ppm.

Estimates of ancient $[CO_2]_{atm}$ can be determined from the equilibrium assemblage of sodium carbonate minerals precipitated from waters in contact with the atmosphere (*6*). At present $[CO_2]_{atm}$ of ~380 ppm, trona ($NaHCO_3$•Na_2CO_3•$2H_2O$) crystallizes at temperatures above ~25°C in at least a dozen modern alkaline saline lakes worldwide. Natron (Na_2CO_3•$10H_2O$) forms at lower temperatures, but nahcolite ($NaHCO_3$) is rare because it is predicted to precipitate only under elevated $[CO_2]$ (Fig. 1A). The preponderance of trona in modern systems indicates that sodium carbonate deposition follows thermodynamic predictions.

During the EECO, long-lived lakes in the western United States deposited oil shale and sodium carbonate evaporites of the Wilkins Peak member of the Green River Formation and equivalents. The dominant sodium carbonate mineral of the Piceance Creek Basin, Colorado, is nahcolite up to ~300 m thick, which in places occurs as microcrystalline chemical mud finely interlayered with halite (NaCl). This nahcolite, confirmed by x-ray diffraction analysis, contains textures diagnostic of precipitation in contact with atmospheric CO_2 at the air-water interface of a perennial lake (fig. S1). The minimum $[CO_2]$ at which pure nahcolite precipitates, determined experimentally, is ~1330 ppm; however, coprecipitation with halite (Fig. 1A) requires a lower minimum $[CO_2]$, anchoring minimum early Eocene $[CO_2]_{atm}$ at >1125 ppm (Fig. 1B) (*6*). Estimates of paleotemperatures from the Green River basin before and after evaporite deposition suggest surface water temperatures varied seasonally from ~20° to 35°C [Supporting Online Material (SOM) text]. Precipitation of nahcolite plus halite at those temperatures fixes minimum early Eocene $[CO_2]_{atm}$ between 1125 and 2985 ppm.

Trona, rather than nahcolite, is the major sodium carbonate in the coeval Green River Formation, Green River Basin, Wyoming. The reaction

$$2NaHCO_3\ (\text{nahcolite}) + 2H_2O + Na^+ \leftrightarrow NaHCO_3 \cdot Na_2CO_3 \cdot 2H_2O\ (\text{trona}) + H^+$$

indicates that trona forms instead of nahcolite under conditions of high activity of Na^+ (a_{Na}^+) and high pH, suggesting different brine chemistries in the Green River and Piceance Creek basin lakes.

The only other economic accumulation of nahcolite is the Anpeng deposit, Henan Province, China, also Eocene in age (SOM text). Trona is the principal sodium carbonate in younger deposits (Fig. 1B), indicating atmospheric $[CO_2]_{atm}$ dropped below 1125 ppm after the Eocene. Atmospheric $[CO_2]$ closer to modern values by the Miocene is suggested by the trona in the Beypazari deposit, Turkey (21.5 Ma). Trona is also the dominant sodium carbonate in the Pleistocene deposits of Searles Lake, California.

Primary nahcolite in the Green River evaporites gives firm geochemical evidence for elevated $[CO_2]_{atm}$ (>1125 ppm) in the early Eocene. These data support a causal connection between CO_2 and global warmth in the EECO and clarify the history of atmospheric CO_2 over the past 60 million years (My) (Fig. 1B). Estimates of early Eocene atmospheric CO_2 from Green River sodium carbonates are in the same range as those predicted by geochemical models (*7*). By ~20 Ma, all available data (*8*) suggest $[CO_2]_{atm}$ was at or near modern concentrations.

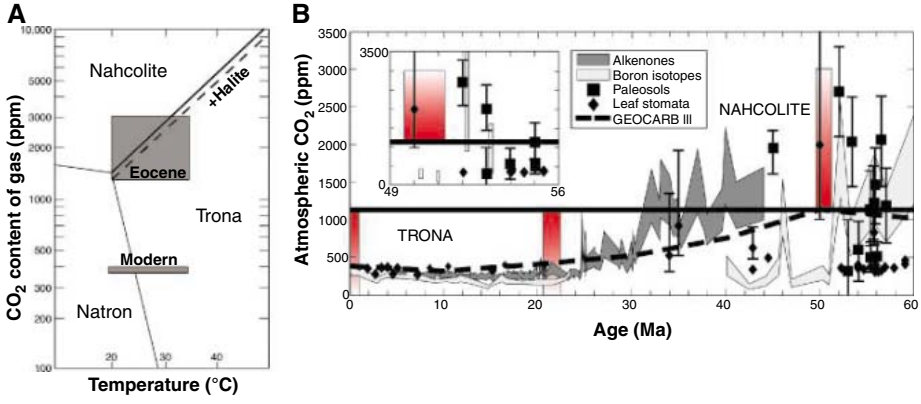

Fig. 1. (**A**) Stability fields of sodium carbonates as a function of $[CO_2]$ and temperature. Minerals are in equilibrium with solution and gas at 1 atm total pressure (*6*). Dashed line shows that addition of halite lowers $[CO_2]$ of the gas in equilibrium with nahcolite plus trona to 1125 ppm at 20°C (*6*). Shaded areas mark environmental boundaries (~20° to 35°C) for precipitation of sodium carbonate minerals today ($[CO_2]_{atm}$ ~ 380 ppm) and during the early Eocene. (**B**) Atmospheric $[CO_2]$ over the past 60 My, estimated from $\delta^{11}B$ of foraminifera (*5*), alkenone $\delta^{13}C$ (*8*), stomatal densities [compilation of (*4*)], paleosol carbonates [(*3*), compilation of (*4*)], and predicted GEOCARB III values (*7*). (Inset) Details for 56 to 49 Ma. The horizontal line at 1125 ppm is the minimum $[CO_2]_{atm}$ necessary to precipitate nahcolite. Probable $[CO_2]_{atm}$ values during deposition of Green River nahcolites (51.3 to 49.6 Ma), Beypazari trona (21.5 Ma ± 0.9 Ma), and Searles Lake trona (<1 Ma) are shaded red. Errors shown are discussed in (*3*–*5*) and (*8*).

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Supporting Online Material

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SOM Text
Fig. S1

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The Connectivity Map: Using Gene-Expression Signatures to Connect Small Molecules, Genes, and Disease

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To pursue a systematic approach to the discovery of functional connections among diseases, genetic perturbation, and drug action, we have created the first installment of a reference collection of gene-expression profiles from cultured human cells treated with bioactive small molecules, together with pattern-matching software to mine these data. We demonstrate that this "Connectivity Map" resource can be used to find connections among small molecules sharing a mechanism of action, chemicals and physiological processes, and diseases and drugs. These results indicate the feasibility of the approach and suggest the value of a large-scale community Connectivity Map project.

A fundamental challenge that arises throughout biomedicine is the need to establish the relation among diseases, physiological processes, and the action of small-molecule therapeutics. Our goal is to provide a generic solution to this problem by attempting to describe all biological states—physiological, disease, or induced with a chemical or genetic construct—in terms of genomic signatures, create a large public database of signatures of drugs and genes, and develop pattern-matching tools to detect similarities among these signatures. Using such a resource, a researcher studying a drug candidate, a gene, or a disease state could compare its signature to the database to discover unexpected connections—much as one can compare a DNA sequence to the GenBank database to identify similar genes. We will refer to this resource as a "Connectivity Map" because of its potential to reveal "connections" among drugs, genes, and diseases.

In principle, there are many possible genomic signatures that might be used—including DNA methylation patterns, mRNA levels, and protein expression or metabolite profiles. To be practical, however, such signatures should be generated from a small number of cells at low cost, in high throughput, and with sufficiently high complexity to provide a rich description. At present, only mRNA expression assayed on DNA microarrays meets these criteria. We have therefore chosen this as the "universal language" with which to describe cellular responses.

Gene-expression profiling has historically been applied in specific settings to elucidate the mechanisms underlying a biological pathway (*1*, *2*), to reveal cryptic subtypes of a disease (*3*, *4*), and to predict cancer prognosis (*5*, *6*). But here we envisage its use as the means to catalog the biological responses to a large number of diverse perturbations. Of course, this idea is not entirely new. A landmark study by Hughes *et al.* (*7*) demonstrated that a compendium of gene-expression data could be used for the functional annotation of small molecules and genes, at least in yeast. Although that study was encouraging, the extent to which the approach would be applicable to mammalian biology was not obvious. More recently, a variety of commercial databases of expression profiles from rat tissues after systemic administration of known drugs have been developed [e.g., (*8*)], and these appear to have value for the identification of potential toxicities of new chemical entities [e.g., (*9*)]. However, such in vivo studies suffer from serious practical limitations. First, the type of perturbagens that can be studied is limited. Only small molecules with druglike physicochemical properties can be effectively administered to live animals. And systematic genetic perturbation (i.e., with RNA interference) is not yet possible. Second, the high cost of whole-animal studies precludes contemplating such an approach at the genome scale.

We hypothesized that perturbations in mammalian cell culture might provide an approach that is truly generalizable, systematic, and biologically relevant. However, several potential pitfalls must be considered. Conceivably, a large number of parameters would need to be optimized for each perturbation, including cell type, concentration, and treatment duration. Equally, analytical methods capable of detecting relevant signals in the data might not be generally applicable. If so, generation of a useful Connectivity Map would be impractical. However, here we demonstrate—through the recovery of known, and the discovery of new, biological connections—that the Connectivity Map concept is indeed viable.

Creating a First-Generation Connectivity Map

Perturbagens. We studied 164 distinct small-molecule perturbagens, selected to represent a broad range of activities, and including U.S. Food and Drug Administration (FDA)–approved drugs and nondrug bioactive "tool" compounds. We included multiple compounds sharing molecular targets (e.g., histone deacetylase inhibitors) to determine whether such compounds would share a molecular signature. Similarly, we profiled compounds with the same clinical indication (e.g., antidiabetics), which allowed us to determine whether connections could be established on the basis of therapeutic class, even though the mechanisms of action might be distinct. Furthermore, we chose some small molecules that act proximal to gene expression (e.g., selective estrogen receptor modulators) and some whose primary targets are much more distal (e.g., immunomodulators, inhibitors of signal transduction). Finally, we included some compounds whose targets are not expressed in all cell types (e.g., COX2 inhibitors), whose clinical effects are non–cell-autonomous (e.g., aromatase inhibitors), or whose activities are only discernible after chronic, in vivo exposure (e.g., antipsychotics).

Cell lines. Ideally, one would generate profiles in a wide diversity of established and primary cells, but practicality limits us to only a few lines that can be stably grown over long periods of time. For this pilot study we generated most of our data in the breast cancer epithelial cell line MCF7 because it has been extensively molecularly characterized, is used as a reference cell line in laboratories throughout the world, and is amenable to culture in microtiter plates. A subset of perturbagens were also profiled in the prostate cancer epithelial cell line PC3 and the nonepithelial lines HL60 (leukemia) and SKMEL5 (melanoma). This diversity of cell types provides an opportunity to assess the extent to which results are context dependent.

Concentration and duration of treatment. High-throughput, cell-based small-molecule screens are often performed at a single, relatively high concentration of 10 μM. We adopted this approach as well, given that the optimal concentration is not known for many compounds of potential interest. For some compounds, we used concentrations reported to be effective in cell

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culture or to approximate the maximum attainable plasma concentrations after therapeutic dosing. We also profiled a subset of compounds across a range of concentrations to explore the sensitivity of results to dose.

As with concentration, the duration of compound treatment might also affect the gene-expression profiles. Profiles obtained too early might not yield robust signals—particularly for perturbations that do not directly modulate transcription—and those obtained too late might reflect secondary and tertiary responses. Because our goal was to obtain signatures related to direct mechanisms of action, we selected a relatively early time point (6 hours after compound addition, with a subset also profiled at 12 hours for comparison).

Control perturbations. Every treatment "instance" was defined relative to a control consisting of cells grown in the same plate and treated with vehicle alone. This approach was taken to minimize the impact of batch-to-batch biological and technical variation. Most of the perturbagens were also profiled multiple times.

Overall data. Our data set was thus composed of genomewide mRNA expression data for 164 distinct bioactive small-molecule perturbagens and corresponding vehicle controls applied to human cell lines for short duration. These data were collected in multiple batches over a period of 1 year by means of Affymetrix GeneChip microarrays. A total of 564 gene-expression profiles were produced, representing 453 individual instances (i.e., one treatment and vehicle pair). Full details of the data set are provided as table S1. The data are freely available for download at www.broad.mit.edu/cmap.

Querying the Connectivity Map

The traditional method for identifying small molecules with similar effects on the basis of gene-expression profiles is hierarchical clustering. Indeed, such a strategy was found to be useful for analyzing data from yeast (*7*) and rat tissues (*10*). However, we saw three drawbacks with such an approach. First, with mammalian cell culture, the dominant structure we detected by hierarchical clustering was related to cell type and batch effects (similarity among cells grown at the same time), and this masked the more subtle signals from short-duration treatment with small molecules (fig. S1). Second, a hierarchical clustering approach would require that all profiles be generated on the same microarray platform, limiting future utility. Third, and most important, we required an analytical method that could detect multiple components within the cellular response to a given perturbation.

For these reasons, we adopted a nonparametric, rank-based pattern-matching strategy based on the Kolmogorov-Smirnov statistic (*11*), as we described previously and later formalized in Gene Set Enrichment Analysis (GSEA) (*2*, *12*, *13*). The approach starts with a "query signature" and assesses its similarity to each of the reference expression profiles in the data set. A query signature is any list of genes whose expression is correlated with a biological state of interest. Examples could include genes correlated with a subtype of disease (e.g., drug-resistant versus drug-sensitive leukemia) or regulated by a biological process of interest (e.g., experimental activation of a signaling pathway). Each gene in the query signature carries a sign, indicating whether it is up-regulated or down-regulated. Because the query signature is unitless, it is not tied to any technology platform.

The reference gene-expression profiles in the Connectivity Map data set are also represented in a nonparametric fashion. Each profile is compared to its corresponding intrabatch vehicle-treated control. The genes on the array are rank-ordered according to their differential expression relative to the control; each treatment instance thus gives rise to a rank-ordered list of ~22,000 genes.

The query signature is then compared to each rank-ordered list to determine whether up-regulated query genes tend to appear near the top of the list and down-regulated query genes near the bottom ("positive connectivity") or vice versa ("negative connectivity"), yielding a "connectivity score" ranging from +1 to −1. A null (zero) connectivity score is assigned where the enrichment scores for the up- and down- regulated genes have the same sign. All instances in the database are then ranked according to their connectivity scores; those at the top are most strongly correlated to the query signature, and those at the bottom are most strongly anticorrelated (Fig. 1). (For expression profiles derived from a single technology platform, we obtained similar results using conventional measures of correlation, such as the Pearson correlation coefficient.)

There is no standard approach for estimating the statistical significance of the connections observed. We therefore resorted to the simplest, most empirical, and most transparent test we could devise, and note that the power to detect connections may be greater for compounds with many replicates. Below, we have focused on cases where the precise calculation of *P*-values is not critical to support our conclusions because the observed connections were so striking (and in several cases validated with functional experiments).

Connections Between Small Molecules

HDAC inhibitors. We first determined whether a query signature derived from a class of small molecules could recover those same compounds in the Connectivity Map. A recent report (*14*) described gene-expression responses of T24 (bladder), MDA 435 (breast carcinoma), and MDA 468 (breast carcinoma) cells treated with three histone deacetylase (HDAC) inhibitors: vorinostat (also known as suberoylanilide hydroxamic acid or SAHA), MS-27-275, and trichostatin A. The authors of this study defined a 13-gene signature (8 up-regulated and 5 down-regulated genes; Signature S1) that was used to query our database.

Despite the differences in the cells used to generate the query signature and reference profiles, the two highest-scoring compounds in the Con-

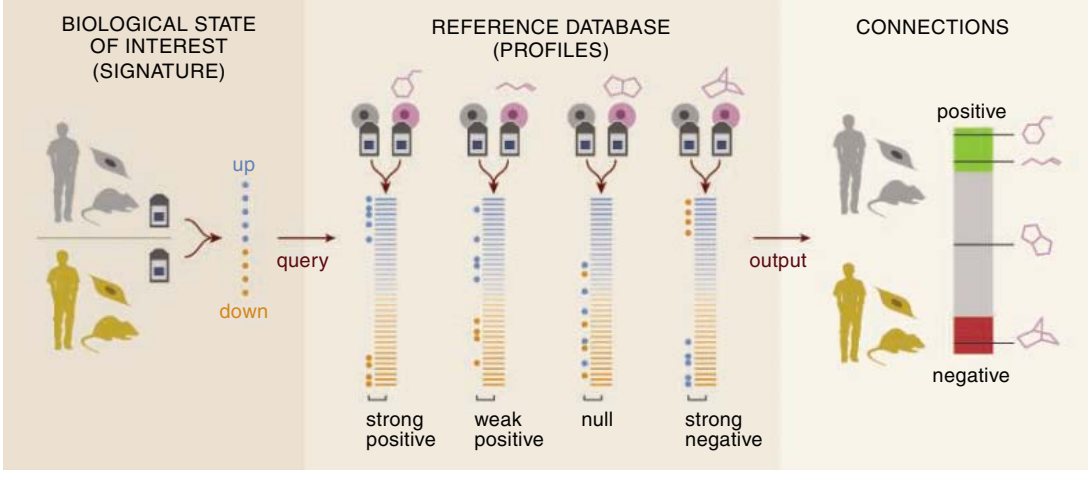


Fig. 1. The Connectivity Map Concept. Gene-expression profiles derived from the treatment of cultured human cells with a large number of perturbagens populate a reference database. Gene-expression signatures represent any induced or organic cell state of interest (**left**). Pattern-matching algorithms score each reference profile for the direction and strength of enrichment with the query signature (**center**). Perturbagens are ranked by this "connectivity score"; those at the top ("positive") and bottom ("negative") are functionally connected with the query state (**right**) through the transitory feature of common gene-expression changes.

nectivity Map were vorinostat and trichostatin A (Fig. 2A). More important, the Connectivity Map also revealed strong connectivity with two structurally distinct compounds, valproic acid (initially developed as an antiseizure drug) and HC toxin, both of which are now known to have HDAC-inhibitory activity but were not used to define the query signature (Fig. 2, A and B). These results indicate that the Connectivity Map would have suggested the HDAC-inhibitory activity of these compounds had it not already been known.

The ability to detect these HDAC inhibitors was not highly sensitive to the precise concentration of drug used to generate the reference profiles. Specifically, the Connectivity Map contains instances of valproic acid at six concentrations (10, 2, and 1 mM; 500, 200, and 50 μM) bracketing the commonly used HDAC-inhibitory level of 1 mM. Only the two lowest concentrations failed to yield a positive connectivity score (Fig. 2A). The results indicate that, at least for this example, connectivity can be established without elaborate optimization of cell type and compound concentration.

Estrogens. We next studied the effects of estrogen, which is known to modulate nuclear hormone signaling. The query signature was taken from a report by an independent group (*15*) in which MCF7 cells were treated with the natural estrogen receptor (ER) ligand, 17β-estradiol (E2). The query signature consisted of 129 genes (40 up- and 89 down-regulated; Signature S2).

The Connectivity Map yielded high positive connectivity scores for all instances of E2 in MCF7 cells. High connectivity scores were also observed for genistein, which is a phytoestrogen (*16*). Weaker connectivity was seen with 17α-estradiol, consistent with its markedly lower affinity for ER than its stereoisomer (*17*) (Fig. 3A).

The Connectivity Map also identified compounds with clear negative connectivity, indicating an opposite effect to that of E2. The highest negative connectivity scores came from fulvestrant, a known anti-estrogenic drug (*18*) (Fig. 3B). Tamoxifen and raloxifene, also anti-estrogens, scored negatively, but to a lesser extent (fig. S2). Together, these results indicate that both agonists and antagonists can be discovered directly from the Connectivity Map.

We used estrogen connectivity to explore the impact of physiological context. Such context is known to be particularly important in the study of ER activity, where growing cells in culture medium containing the estromimetic phenol red and supplemented with complete serum (which contains endogenous estrogens) often obscures estrogen stimulation signals as measured with traditional read-outs such as reporter assays or gel shifts. We therefore asked whether the 129-gene estrogen signature might be more robust to the particulars of culture medium composition. Indeed, the presence of phenol red and complete serum had little effect on connectivity scores for E2, even though the query signature was defined under estrogen-free conditions (*15*). Indeed, the connectivity scores were similar to those made in phenol red-free medium with charcoal-stripped serum (ssMCF7; Fig. 3A). However, the anti-estrogen fulvestrant received a null connectivity score in MCF7 cells under estrogen-free conditions, consistent with its "pure antagonist" mode of action (Fig. 3B). Similarly, no robust estrogenic or anti-estrogenic connections were recovered in treatments performed in PC3 or HL60 cells, neither of which expresses ER. These results indicate that although gene-expression signatures can be highly sensitive, some connections will not be found if the reference profiles are collected in cells that lack the appropriate physiological or molecular context.

Phenothiazines. We next considered small molecules that do not directly regulate gene expression. We studied the phenothiazine antipyschotics, which as dopamine receptor antagonists and calmodulin inhibitors are not immediately proximal to transcription. Reference profiles were generated for five phenothiazines (chlorpromazine, fluphenazine, prochlorperazine, thioridazine, trifluoperazine) representing three structural subclasses (Fig. 4A). At least three instances of each were produced, mainly in MCF7 cells and at a concentration of 10 μM, with the exception of chlorpromazine, which was profiled three times at 1 μM and only once at 10 μM.

For these experiments, query signatures were generated from a subset of the reference profiles within the Connectivity Map data set itself. A query signature consisting of genes consistently regulated across one instance of each of the five phenothiazines was first established (Signature S3). We then used this signature to assess the recovery of all of the remaining phenothiazine instances from our database. As anticipated, the five instances used to derive the signature received the highest connectivity scores. More important, 10 of the 13 nonsignature instances were also highly ranked (Fig. 4B). The three instances not

rank	perturbagen	dose	cell	score
1	vorinostat [1000]	10 μM	MCF7	1
2	trichostatin A [873]	1 μM	MCF7	0.969
3	trichostatin A [992]	100 nM	MCF7	0.931
4	trichostatin A [1050]	100 nM	MCF7	0.929
5	vorinostat [1058]	10 μM	MCF7	0.917
6	trichostatin A [981]	1 μM	MCF7	0.915
7	HC toxin [909]	100 nM	MCF7	0.914
8	trichostatin A [1112]	100 nM	MCF7	0.908
9	trichostatin A [1072]	1 μM	MCF7	0.906
10	trichostatin A [1014]	1 μM	MCF7	0.893
11	trichostatin A [332]	100 nM	MCF7	0.882
12	trichostatin A [331]	100 nM	MCF7	0.846
13	trichostatin A [448]	100 nM	PC3	0.788
14	valproic acid [345]	10 mM	MCF7	0.743
15	valproic acid [23]	1 mM	MCF7	0.735
16	valproic acid [1047]	1 mM	MCF7	0.733
17	trichostatin A [413]	100 nM	ssMCF7	0.725
18	valproic acid [410]	10 mM	HL60	0.725
19	valproic acid [458]	1 mM	PC3	0.680
33	valproic acid [409]	1 mM	HL60	0.634
39	valproic acid [1020]	500 μM	MCF7	0.619
52	valproic acid [346]	2 mM	MCF7	0.582
61	valproic acid [1078]	500 μM	MCF7	0.563
71	valproic acid [629]	1 mM	SKMEL5	0.539
72	valproic acid [347]	500 μM	MCF7	0.539
73	valproic acid [989]	1 mM	MCF7	0.538
76	valproic acid [433]	1 mM	PC3	0.528
89	trichostatin A [364]	100 nM	HL60	0.507
92	valproic acid [497]	1 mM	ssMCF7	0.501
297	valproic acid [348]	50 μM	MCF7	0
388	valproic acid [994]	200 μM	MCF7	0
403	valproic acid [1002]	50 μM	MCF7	0
419	valproic acid [1060]	50 μM	MCF7	-0.537

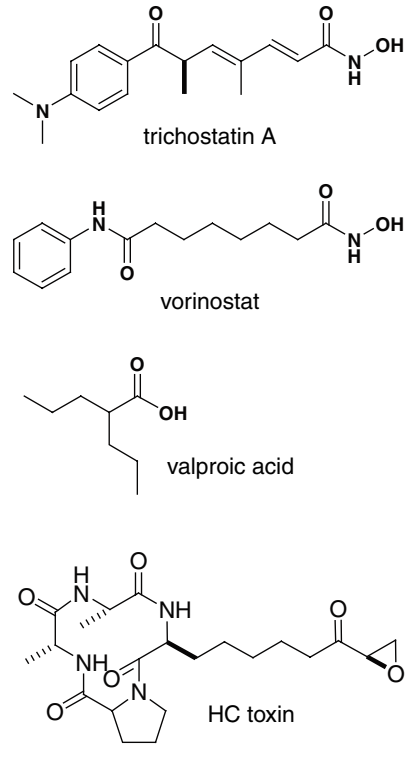


Fig. 2. HDAC Inhibitors. (**A**) HDAC inhibitors are highly ranked with an external HDAC inhibitor signature. The "bar-view" is constructed from 453 horizontal lines, each representing an individual treatment instance, ordered by their corresponding connectivity scores with the Glaser *et al*. (*14*) signature (+1, top; −1, bottom). All valproic acid (n = 18), trichostatin A (n = 12), vorinostat (n = 2), and HC toxin (n = 1) instances in the data set are colored in black. Colors applied to the remaining instances reflect the sign of their scores (green, positive; gray, null; red, negative). The rank, name [instance id], concentration, cell line, and connectivity score for each of the selected HDAC inhibitor instances is shown. Unabridged results from this query are provided as Result S1. (**B**) Chemical structures.

receiving high connectivity scores were the low-concentration chlorpromazine treatments; these therefore served as useful specificity controls. Similar results were obtained with signatures produced from different phenothiazine instances and with different gene-selection criteria (Signatures S4 to S6; fig. S3). These results show that the common activity of these phenothiazine antipsychotic compounds can be recovered by the Connectivity Map, even when analyzed in nonneural cells. They also demonstrate that the approach is not unduly sensitive to signature-definition parameters.

The phenothiazine query signature did not show strong connectivity with the nonphenothiazine antipsychotics haloperidol and clozapine (Fig. 4C). This is not surprising because, although these antipsychotics ultimately target the same neurotransmitter receptors, the receptors themselves are not expressed in the cell lines used. Indeed, the antipsychotics were included in this data set as an extreme test of the Connectivity Map concept.

The analysis of the phenothiazine query signature did yield consistently strong negative connectivity scores for arachidonic acid (Fig. 4C). Arachidonic acid is the primary substrate for cyclooxygenases and lipoxygenases and is thus a critical precursor for both prostaglandin and leukotreine syntheses. The Connectivity Map result suggests that phenothiazines have an activity that mimics ablation of the arachidonic acid cascade and is therefore entirely consistent with the observation that phenothiazines can inhibit prostaglandin synthesis (*19*). Indeed, more recently, phenothiazine derivatives have been developed as potent dual cyclooxygenase/lipoxygenase inhibitors that exhibit anti-inflammatory activity (*20*). Had this activity of phenothiazines not been previously discovered by serendipity, it would have been systematically revealed by the Connectivity Map.

These findings confirm that even perturbagens not acting immediately proximal to transcription do give rise to distinguishable gene-expression profiles and demonstrate again that the Connectivity Map can reveal complex biological activities. They also show that the Connectivity Map approach can use both internal as well as external query signatures.

Identification of gedunin as an HSP90 inhibitor. We next sought to use the Connectivity Map to generate hypotheses about the mechanism of action of an uncharacterized small molecule. In a separate study, we performed a high-throughput gene expression–based screen for small molecules capable of abrogating the gene-expression signature of androgen receptor (AR) activation in prostate cancer cells. The details of the screen and its biochemical follow-up are described elsewhere (*21*). One of the hits from the screen was the triterpenoid natural product gedunin (*22*) (Fig. 5A), purified from the *Meliacae* family of medicinal plants. The mechanism by which gedunin abrogated AR activity was entirely unknown because this compound has not been extensively characterized.

In an effort to elucidate its mechanism of action, we defined a signature for gedunin (Signature S7) by treating LNCaP prostate cancer cells for 6 hours with the compound, and queried the Connectivity Map. High connectivity scores were found for multiple instances of three heat shock protein 90 (HSP90) inhibitors: geldanamycin, 17-allylamino-geldanamycin, and 17-dimethylamino-geldanamycin (Fig. 5B). As a class, these HSP90 inhibitors showed marked connectivity to the gedunin signature (permutation P-value < 0.0001).

This result suggests that gedunin, though structurally dissimilar from known HSP90 inhibitors (Fig. 5A), might impinge upon the HSP90 pathway. Because the stability of AR is known to be dependent upon HSP90 activity, we asked whether AR expression could be diminished by gedunin treatment. Immunoblotting indicated that AR protein, as well as other HSP90-interacting proteins, was nearly entirely eliminated in gedunin-treated LNCaP and Ba/F3 cells (Fig. 5C), consistent with gedunin acting as an inhibitor of HSP90 function. Moreover, mutant interacting proteins such as the BCR-ABL T315I point mutant and the FLT3 internal tandem duplication (ITD) mutant show increased sensitivity to gedunin-mediated inhibition, as is seen upon HSP90 inhibition by geldanamycins (*23*, *24*). Further biochemical studies demonstrated that the mechanism of abrogating HSP90 function was distinct from geldanamycin and its analogs (*21*).

These experiments demonstrate that the Connectivity Map can generate testable hypotheses about the target pathways of poorly characterized small molecules, providing a potentially powerful tool for pharmaceutical development.

Connections with Disease States

We next sought to collect query signatures from disease states and scan the Connectivity Map to identify small molecules that might mimic or suppress that disease.

Diet-induced obesity. We made use of a signature for the obese state from a published report (*25*) of the genes differentially expressed in a rat model of diet-induced obesity (Signature S8). The conditions used in that study differed sharply from those used to build the Connectivity Map with respect to RNA source (adipose tissue versus cell lines), treatment duration (65 days versus 6 hours), and species (rat versus human). Despite these differences, instances of three peroxisome proliferator-activated receptor gamma (PPARγ) agonists—the thiazolidindiones (TZD), troglitazone and rosiglitazone, and indometacin (*26–28*)—received high connectivity scores (fig. S4). Indeed, all three compounds are potent inducers of adipogenesis in vitro (*26–28*). Further, that TZDs promote weight gain in vivo has been widely observed as a consequence of their clinical use

A

rank	perturbagen	dose	cell	score
2	estradiol [988]	100 nM	MCF7	0.936
3	estradiol [373]	10 nM	ssMCF7	0.918
4	genistein [1015]	10 μM	MCF7	0.913
5	estradiol [1079]	10 nM	MCF7	0.899
6	estradiol [1021]	10 nM	MCF7	0.813
8	alpha-estradiol [990]	10 nM	MCF7	0.809
9	alpha-estradiol [403]	10 nM	ssMCF7	0.807
10	estradiol [414]	10 nM	ssMCF7	0.794
11	estradiol [121]	10 nM	MCF7	0.758
12	genistein [1073]	10 μM	MCF7	0.753
13	genistein [638]	10 μM	MCF7	0.730
17	alpha-estradiol [1048]	10 nM	MCF7	0.646
20	genistein [268]	1 μM	MCF7	0.619
21	estradiol [365]	100 nM	MCF7	0.610
25	genistein [382]	10 μM	MCF7	0.561
27	genistein [267]	1 μM	MCF7	0.552
46	alpha-estradiol [122]	10 nM	MCF7	0.435
51	estradiol [387]	10 nM	HL60	0.421
64	estradiol [782]	10 nM	HL60	0.376
148	alpha-estradiol [702]	10 nM	PC3	0
152	genistein [703]	10 μM	PC3	0
162	alpha-estradiol [762]	10 nM	MCF7	0
278	estradiol [665]	10 nM	PC3	0

B

rank	perturbagen	dose	cell	score
171	fulvestrant [704]	1 μM	PC3	0
261	fulvestrant [523]	1 μM	ssMCF7	0
447	fulvestrant [367]	1 μM	MCF7	-0.749
450	fulvestrant [310]	10 nM	MCF7	-0.843
451	fulvestrant [985]	1 μM	MCF7	-0.961
452	fulvestrant [1076]	10 nM	MCF7	-0.989
453	fulvestrant [1043]	1 μM	MCF7	-1

Fig. 3. Estrogen receptor agonists and antagonists. (**A**) Estrogen receptor agonists are highly ranked with an external 17β-estradiol signature. Barview (as in Fig. 2) with all estradiol (n = 10), alpha-estradiol (n = 7), and genistein (n = 7) instances shown. (**B**) A pure antiestrogen is negatively connected. Barview with all fulvestrant (n = 7) instances shown. Unabridged results from this query are provided as Result S2.

as oral antidiabetic agents and is considered a major drawback of their use (*29*). The Connectivity Map would have predicted this particular adverse effect.

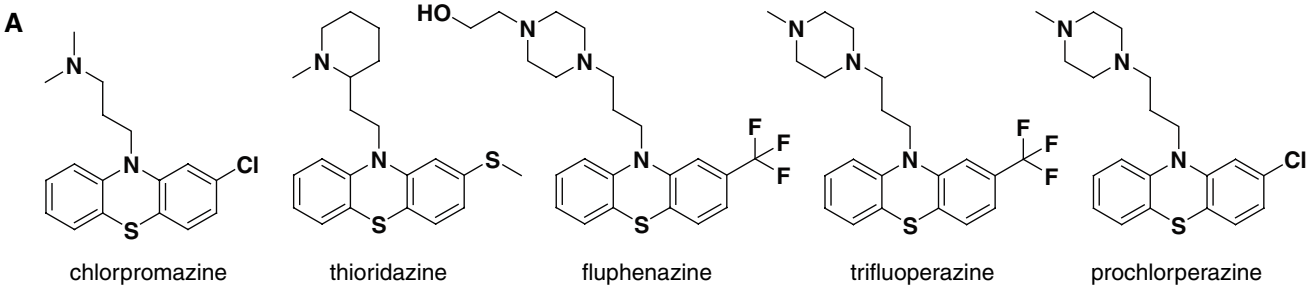
These results must be tempered, however, because they derive solely from PC3—the only cell line in our panel to express PPARγ at high levels (*30*)—and TZD and indometacin instances made in all other cell lines yielded null or negative scores. Clearly, these connections would not have been made had this particular cellular context not been represented. Of note, the other known PPARγ agonist in our collection, 15-delta prostaglandin J2 (*26*), received a null score even in PC3, although the entire set of agonists (including 15-delta prostaglandin J2) still showed significant enrichment as a class (permutation *P*-value = 0.0021). Overall, it is notable that a signature derived from rat adipose tissue after many weeks of treatment can generate connections with small molecules applied acutely to epithelial cells in culture.

Alzheimer's disease. We next explored query signatures for Alzheimer's disease (AD). AD is the most common cause of dementia in the elderly, but its pathogenesis is poorly understood and effective therapies remain elusive. We made use of two independent reports of the gene-expression changes in brain tissue from AD patients.

The first signature consisted of 40 genes identified through a comparison of hippocampus from AD and normal brain (*31*) (Signature S9). The second, derived from the comparison between cerebral cortex from AD brain and age-matched controls, contained 25 genes (*32*) (Signature S10). Although there were no genes in common between these two query signatures, both yielded statistically significant negative connectivity with the two independent instances of 4,5-dianilinophthalimide (DAPH) in the Connectivity Map (fig. S5). No other compound in the database shared this behavior.

DAPH was recently identified in a cell-free screen for small molecules that could reverse the formation of fibrils (specifically, decreasing the β-sheet content of aggregating Aβ1-42 peptide) thought to be responsible for the accelerated neuronal cell death in the brains of AD patients (*33*). Indeed, a variety of new DAPH analogs have since been synthesized as potential treatments for AD (*34*). Our observations strengthen the candidacy of DAPH as a potential AD therapeutic and further illustrate the potential of the Connectivity Map to generate novel, unbiased hypotheses concerning the pharmacologic modulation of disease states.

Dexamethasone resistance in ALL. As a final example, we considered one of the most vexing problems in cancer chemotherapy: drug resistance. Specifically, we explored resistance to the glucocorticoid dexamethasone in children with acute lymphoblastic leukemia (ALL). Dexamethasone resistance has been observed both in vivo and in primary leukemia cells grown in short-term culture (*35*). We defined a gene-expression signature of dexamethasone sensitivity (Signature S11) by comparing bone-marrow leukemic cells from patients exhibiting either dexamethasone sensitivity or resistance in vitro. The details of this signature are reported elsewhere (*36*).



B

rank	perturbagen	dose	cell	score
1	trifluoperazine [421]	10 μM	MCF7	1
2	prochlorperazine [455]	10 μM	MCF7	0.972
3	fluphenazine [490]	10 μM	MCF7	0.957
4	thioridazine [417]	10 μM	MCF7	0.951
5	chlorpromazine [419]	10 μM	MCF7	0.926
7	thioridazine [422]	1 μM	MCF7	0.628
8	trifluoperazine [910]	10 μM	MCF7	0.621
9	thioridazine [1068]	10 μM	MCF7	0.620
10	prochlorperazine [1053]	10 μM	MCF7	0.616
11	thioridazine [1010]	10 μM	MCF7	0.602
12	trifluoperazine [1004]	10 μM	MCF7	0.601
14	fluphenazine [1075]	10 μM	MCF7	0.590
17	prochlorperazine [995]	10 μM	MCF7	0.571
21	fluphenazine [1017]	10 μM	MCF7	0.542
58	fluphenazine [494]	10 μM	SKMEL5	0.426
256	chlorpromazine [426]	1 μM	MCF7	0
332	chlorpromazine [997]	1 μM	MCF7	0
342	chlorpromazine [1055]	1 μM	MCF7	0

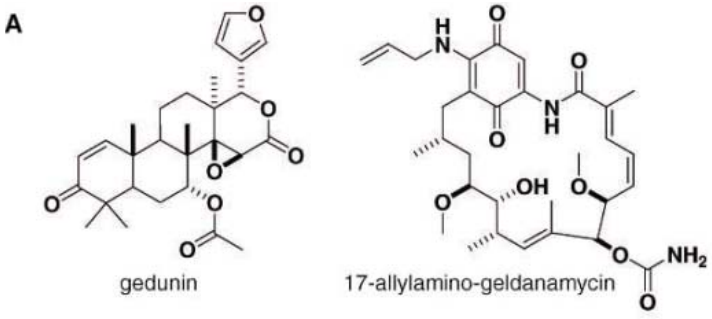
C

rank	perturbagen	dose	cell	score
6	haloperidol [418]	10 μM	MCF7	0.753
15	clozapine [416]	10 μM	MCF7	0.580
22	haloperidol [492]	10 μM	MCF7	0.541
27	haloperidol [983]	10 μM	MCF7	0.524
80	clozapine [1009]	10 μM	MCF7	0.385
127	haloperidol [1041]	10 μM	MCF7	0.314
216	haloperidol [1024]	10 μM	MCF7	0
257	haloperidol [1082]	10 μM	MCF7	0

rank	perturbagen	dose	cell	score
427	arachidonic acid [443]	10 μM	MCF7	-0.602
442	arachidonic acid [604]	10 μM	MCF7	-0.843
451	arachidonic acid [441]	10 μM	MCF7	-0.971

Fig. 4. Phenothiazine connections. (**A**) Chemical structures. Three structural subclasses are shown: with a piperazine group in the side chain (fluphenazine, trifluoperazine, prochlorperazine), with a piperidine ring in the side chain (thioridazine), and with an aliphatic side chain (chlorpromazine). (**B**) Recovery of phenothiazine instances with an internal phenothazine signature. Barview (as in Fig. 2) with all thioridazine (n = 4), chlorpromazine (n = 4), fluphenazine (n = 4), trifluoperazine (n = 3), and prochlorperazine (n=3) instances shown. The instances used to generate the signature are shaded. (**C**) Ranking of nonphenothiazine antipsychotics and arachidonic acid instances with the phenothiazine signature. Barview showing all haloperidol (n = 6), clozapine (n = 2), and arachidonic acid (n = 3) instances. Permutation *P*-values are 0.1428, 0.0621, and 0.0002, respectively. Unabridged results from this query are provided as Result S3.

When the signature of dexamethasone sensitivity was used to query the Connectivity Map, we found strong connectivity to the mTOR inhibitor sirolimus (also known as rapamycin) (Fig. 6A). This result suggested that sirolimus might revert dexamethasone resistance. Indeed, treatment of the lymphoid cell line CEM-c1 with sirolimus conferred dexamethasone sensitivity to this otherwise resistant cell line, reducing the median inhibitory concentration (IC_{50}) by a factor of more than 50 (Fig. 6B). Additional experiments indicated that this activity was mTOR dependent, resulting in apoptosis mediated through down-regulation of the anti-apoptotic protein MCL1 (*36*). Whatever the mechanism, the result from



B

rank	perturbagen	dose	cell	score
5	17-allylamino-geldanamycin [1064]	1 μM	MCF7	0.893
8	17-allylamino-geldanamycin [947]	1 μM	MCF7	0.866
9	17-allylamino-geldanamycin [1063]	1 μM	MCF7	0.858
12	geldanamycin [972]	1 μM	MCF7	0.837
13	17-allylamino-geldanamycin [1056]	1 μM	MCF7	0.836
14	17-allylamino-geldanamycin [831]	1 μM	MCF7	0.830
15	17-allylamino-geldanamycin [1044]	1 μM	MCF7	0.822
17	17-dimethylamino-geldanamycin [1051]	100 nM	MCF7	0.808
18	geldanamycin [1008]	1 μM	MCF7	0.787
19	geldanamycin [1066]	1 μM	MCF7	0.784
20	17-allylamino-geldanamycin [916]	1 μM	MCF7	0.769
21	17-allylamino-geldanamycin [998]	1 μM	MCF7	0.767
23	17-allylamino-geldanamycin [986]	1 μM	MCF7	0.748
25	17-allylamino-geldanamycin [1006]	1 μM	MCF7	0.725
27	geldanamycin [611]	1 μM	MCF7	0.710
28	17-allylamino-geldanamycin [1005]	1 μM	MCF7	0.699
30	geldanamycin [864]	1 μM	MCF7	0.686
33	17-allylamino-geldanamycin [428]	1 μM	MCF7	0.654
34	17-dimethylamino-geldanamycin [993]	100 nM	MCF7	0.651
37	geldanamycin [593]	1 μM	MCF7	0.639
48	17-allylamino-geldanamycin [521]	1 μM	ssMCF7	0.571
54	17-allylamino-geldanamycin [381]	1 μM	MCF7	0.553
60	17-allylamino-geldanamycin [221]	100 nM	MCF7	0.540
71	17-allylamino-geldanamycin [505]	1 μM	SKMEL5	0.517
74	17-allylamino-geldanamycin [432]	1 μM	PC3	0.504
87	17-allylamino-geldanamycin [450]	1 μM	PC3	0.484

C

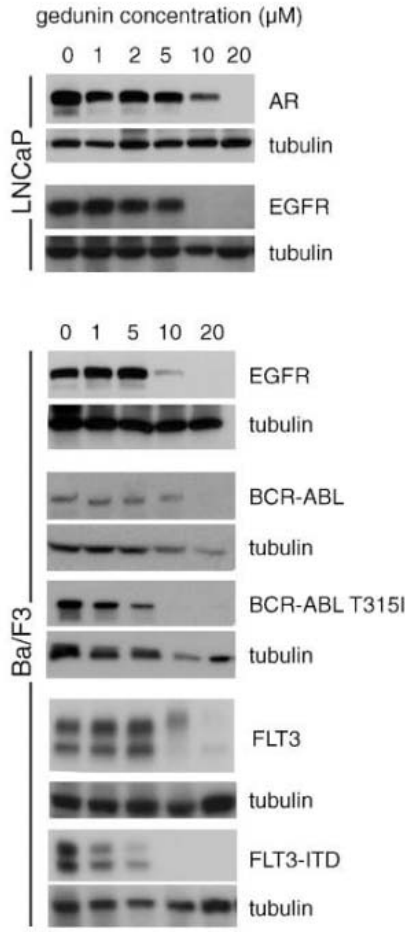


Fig. 5. Gedunin modulates the HSP90 pathway. (**A**) Chemical structure of gedunin and 17-allylamino-geldanamycin. (**B**) Gedunin is connected with geldanamycin and its analogs. Barview (as in Fig. 2) showing all 17-allylamino-geldanamycin (n = 18), geldanamycin (n = 6), and 17-dimethylamino-geldanamycin (n = 2) instances for the gedunin signature. Unabridged results from this query are provided as Result S7. (**C**) Gedunin lowers the levels of HSP90-interacting proteins, including the androgen receptor (AR), in LNCaP cells and Ba/F3 cells ectopically expressing them. Mutant HSP90-interacting proteins (BCR-ABL T315I point mutant and the FLT3-ITD internal tandem duplication mutant) show increased sensitivity to gedunin treatment. EGFR, epidermal growth factor receptor.

A

rank	perturbagen	dose	cell	score
1	sirolimus [1059]	100 nM	MCF7	1
2	sirolimus [987]	100 nM	MCF7	0.979
7	sirolimus [1045]	100 nM	MCF7	0.930
11	sirolimus [1022]	100 nM	MCF7	0.897
16	sirolimus [1080]	100 nM	MCF7	0.880
30	sirolimus [1001]	100 nM	MCF7	0.810
35	sirolimus [402]	100 nM	ssMCF7	0.793
39	sirolimus [362]	100 nM	HL60	0.778
64	sirolimus [921]	100 nM	MCF7	0.687
104	sirolimus [326]	100 nM	MCF7	0.530

B

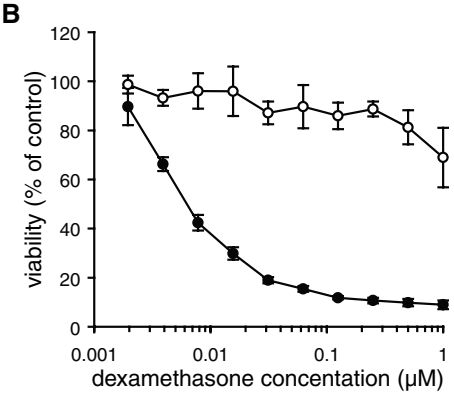


Fig. 6. Sirolimus reverses glucocorticoid resistance in acute lymphoblastic leukemia. (**A**) Barview (as in Fig. 2) showing all 10 sirolimus instances. Permutation P-value for this set of instances is <0.0001. Unabridged results from this query are provided as Result S11. (**B**) The effect of a combination of sirolimus and dexamethasone on the viability of glucocorticoid-resistant lymphoid cells. CEM-c1 cells were treated with 10 nM sirolimus and various concentrations of dexamethasone (closed circles) or dexamethasone alone (open circles) for 72 hours. Cell viability was assessed by MTT reduction and expressed relative to untreated control cultures. Plot shows means and standard deviations for triplicate determinations from one representative experiment.

the Connectivity Map immediately suggests that sirolimus should be tested in a clinical trial of ALL patients with dexamethasone resistance. Sirolimus is already FDA approved as an immunosuppressant and is well tolerated in children, and the clinical prognosis of dexamethasone-resistant ALL is poor (*37*–*40*). This example demonstrates that the Connectivity Map is one approach to the rapid identification of new potential uses for existing drugs.

Discussion

The value of a Connectivity Map depends on many open questions. How many distinct cellular pathways and states actually exist? How many cell types must be studied to provide sufficient diversity? How many perturbagens (small molecules, inhibitory RNAs, open reading frames) would need to be characterized to provide substantial coverage? How many concentrations, time points, and replicates would be required to provide reliable data? What analytical tools will be needed to interpret the data and determine precise estimates of statistical significance and false-positive rates? And, most important, what will be the biomedical value of the data? Only empirical evidence will resolve these issues.

Although only a first step, our results are encouraging. They show that genomic signatures can be used to recognize drugs with common mechanisms of action (HDAC inhibitors and estrogen receptor modulators), discover unknown mechanisms of actions (gedunin as an HSP90 inhibitor), and identify potential new therapeutics (the ability of sirolimus to overcome dexamethasone resistance in ALL). Our findings also reveal that signatures are often conserved across diverse cell types and settings (the signature of dexamethasone resistance was defined in bone-marrow samples but searched against profiles from the MCF7 breast cancer line). At the same time, the results demonstrate the limitations of using only a few cell lines (the signature of estradiol was not detected in cells that lack estrogen receptors) or only a few concentrations (chlorpromazine was not recognized as a phenothiazine at 1 μM). It is also likely that our methodologies can still be refined. Indeed, alternative signature-based pattern-matching methods have been developed [e.g., (*41*)]. In addition, the interpretation of results depends on the ability to confidently call connections. More rigorous methods for the estimation of statistical significance are therefore probably also required, especially as the size of the database grows. But overall, the basic features of our approach appear to work well. We have, therefore, created a Web-based tool (www.broad.mit.edu/cmap) to allow researchers to perform their own Connectivity Map analyses with user-defined signatures in real time.

On the basis of the results of this pilot study, we propose that a sensible next step would be the generation of an expanded Connectivity Map as a community resource project in the spirit of other genomic efforts. An initial goal might be to profile all FDA-approved drugs and inhibitory RNAs targeting a large collection of genes in perhaps 10 diverse cell lines. Further goals would depend on the utility of the data. Ultimately, it will be interesting to explore whether it is possible to create a truly comprehensive catalog that begins to saturate all possible cellular states. In the meanwhile, even an incomplete Connectivity Map will likely accelerate progress in characterizing new chemical entities, finding new uses for existing drugs, and understanding the molecular mechanisms of disease.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5795/1929/DC1
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Figs. S1 to S5
Table S1
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Results S1 to S11
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Structure of the 70*S* Ribosome Complexed with mRNA and tRNA

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The crystal structure of the bacterial 70*S* ribosome refined to 2.8 angstrom resolution reveals atomic details of its interactions with messenger RNA (mRNA) and transfer RNA (tRNA). A metal ion stabilizes a kink in the mRNA that demarcates the boundary between A and P sites, which is potentially important to prevent slippage of mRNA. Metal ions also stabilize the intersubunit interface. The interactions of E-site tRNA with the 50*S* subunit have both similarities and differences compared to those in the archaeal ribosome. The structure also rationalizes much biochemical and genetic data on translation.

A major breakthrough for our mechanistic understanding of translation was achieved some years ago when high-resolution structures of the 50*S* and 30*S* ribosomal subunits were solved (*1*, *2*). Progress has also been made in obtaining structural data on the whole ribosome. The subunit structures were used to facilitate interpretation of maps at 5.5 Å resolution of the whole 70*S* ribosome complexed with mRNA and tRNA (*3*). More recently, the structure of the *Escherichia coli* ribosome was solved at 3.5 Å resolution (*4*). At the same

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time, electron cryomicroscopy (cryo-EM) studies have yielded increasingly detailed structures of various functional states of the ribosome (*5*).

Important as these results are, current structural data on whole ribosomes have limitations. At resolutions lower than 3.5 to 4 Å, it is possible to model known structures into maps but difficult to interpret previously unknown regions in molecular terms. For example, in the 5.5 Å crystal structure of the 70*S* ribosome, an attempt was made to interpret the L1 and L7/L12 stalks, which consist of both proteins and RNA (*3*). These interpretations were at variance with high-resolution structures of the components determined later (*6*, *7*). Typical cryo-EM maps of the ribosome have even lower resolution and thus suffer the same limitations. The 3.5 Å resolution crystal structure of the empty *E. coli* ribosome (*4*) allowed many molecular details to be seen directly. However, this structure lacks direct information about the interactions of the ribosome with its mRNA and tRNA ligands.

We report here the structure of a pretranslocation state of the *Thermus thermophilus* ribosome at 2.8 Å resolution, which has allowed us to build an accurate model that reveals the structures of tRNA and mRNA in situ and the molecular details of their interaction with the ribosome.

Crystallization and structure determination. A pretranslocation complex of the ribosome was formed by complexing it with mRNA, deacylated initiator $tRNA^{fMet}$ in the P site, and aminoacyl $tRNA^{Phe}$ in the A site (*8*). The antibiotic paromomycin, which is known both to increase the affinity of A-site tRNA and to inhibit translocation, was also included.

An extensive search for well-diffracting crystals led to a new crystal form grown in a mixture of 3.5 to 4.5% (w/v) PEG20K and 3.5 to 4.5% (v/v) PEG550MME at pH = 7. The crystals were in space group $P2_12_12_1$ (cell dimensions of a = 214 Å, b = 454 Å, c = 630 Å, and $\alpha = \beta = \gamma = 90°$) and contained two copies of the 70*S* ribosome in the asymmetric unit. A molecular replacement solution was found by using a model of the *T. thermophilus* ribosome (*9*) that was derived from atomic models of the 30*S* (*2*) and 50*S* (*1*, *10*) ribosomal subunits. All ligands were removed from the search model before its use. This solution was used as a starting point to build the structure and refine it to 2.8 Å resolution. The mRNA, tRNA, and antibiotic ligands were only included in the final rounds of refinement, so that they could be built into unbiased difference Fourier maps (*8*). A summary of crystallographic data and refinement statistics is given (table S1).

After refinement, the tRNA and mRNA ligands, as well as differences from the input model, could clearly be seen in difference Fourier maps (Fig. 1). It was easily possible to distinguish between purines and pyrimidines, and well-ordered side chains of proteins were clearly visible. It was also possible to see a large number of metal ions.

The current structure consists of nearly the entire 70*S* ribosome with its tRNA and mRNA ligands (fig. S1). The L7/L12 stalk, along with its base consisting of the L10, L11, and L6 regions as well as the elbow and acceptor arm of A-site tRNA, was poorly ordered or disordered. The E site was occupied with a noncognate tRNA.

In the following description of the structure, we focus mainly on those details that have not been seen directly in previous work. These include details of the interactions of tRNA and mRNA with the ribosome and the participation of proteins and metal ions, especially in intersubunit bridges. For the sake of conciseness, we refer to the ribosomal subunits as 30*S* and 50*S*, and to the whole ribosome as 70*S*.

Overall structure. The two molecules in the asymmetric unit are nearly identical, in contrast to the two ribosomes in the asymmetric unit of the *E. coli* 70*S* crystals (*4*). Surprisingly, even mobile regions such as the L1 stalk of the 50*S* are in similar conformations. Thus, binding of mRNA and tRNA confers conformational homogeneity to the ribosome.

The structure of the 50*S* is very similar to that of both 50*S* molecules in the asymmetric unit of the *E. coli* 70*S* crystals (*4*). The only significant difference is that the regions surrounding the E site are further apart in the empty ribosome but move toward each other to bind E-site tRNA (fig. S2). The most notable movement is that of the L1 stalk. Differences in the L1 stalk conformation have also been observed between empty 50*S* and previous 70*S* structures containing E-site tRNA (*1*, *3*, *10*).

In contrast, the 30*S* subunit of the 70*S* complex reported here is more similar to the closed 30*S* form described previously, as a consequence of A-site tRNA binding (*11*), than to either of the two 30*S* molecules of the empty *E. coli* ribosome (*4*), which are both more open to varying degrees (fig. S3). A comparison of the rmsd and median distance of equivalent phosphorus atoms for the subunits from various 70*S* structures is shown in table S2.

An *E. coli* numbering for *Thermus* ribosomal RNA (rRNA) based on structural alignment with the *E. coli* ribosome (*4*) is used throughout. We also used the standard Brimacombe helix numbering for rRNA, with a lowercase h prefix for 16*S* RNA and an uppercase H for 23*S* RNA.

Interactions with tRNA and mRNA. In the hybrid states model, tRNAs move first relative to the 50*S* subunit and then with respect to the 30*S* subunit during translocation (*12*). Thus

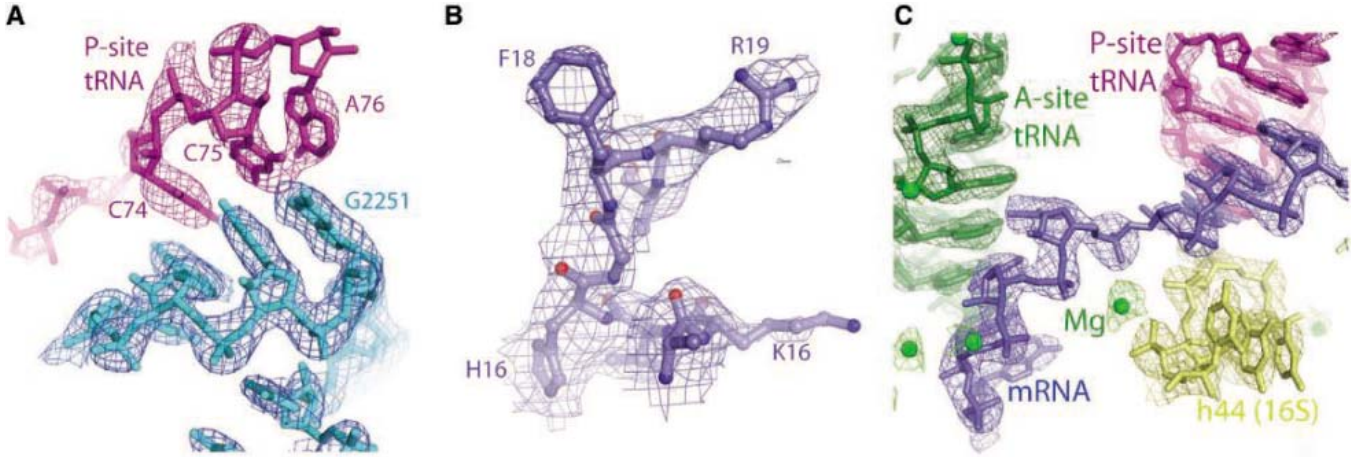

Fig. 1. Representative electron density in the 70*S* structure. (**A**) $F_o - F_c$ map showing unbiased density of the acceptor end of P-site tRNA (not included in the refinement), with refined $3F_o - 2F_c$ density for 23*S* RNA. Base pairs between C74 and C75 with G2252 and G2251 of 23*S* RNA are clearly visible. (**B**) Simulated annealing omit map of the large subunit protein L34 showing the visibility of well-ordered side chains. (**C**) A Mg ion shown in unbiased difference Fourier maps that is coordinated to the nonbridging phosphate oxygens at the 45° mRNA kink between the A and P site as well as the nonbridging phosphate oxygens of nucleotides 1400 and 1401 of h44 of 16*S* RNA. All RNA is shown in final $3F_o - 2F_c$ density. The Mg ions are shown in green. This and all other figures were made with Pymol (*47*).

during the intermediate step, a tRNA could be in the P site of the 30*S* and the E site of the 50*S*, or in the A site of the 30*S* and the P site of the 50*S* (designated as P/E or A/P states). In the structure described here, the tRNAs were in the classical P/P or E/E states, as would be expected for a state before translocation.

The A site. The A site of the ribosome is where codon-anticodon interactions are monitored in the decoding site of the 30*S* subunit and where the new amino acid at the 3′ end of tRNA is made available for peptide bond formation in the peptidyl transferase center (PTC) of the 50*S* subunit. The anticodon stem loop (ASL), comprising nucleotides 26 to 44, was clearly visible in the decoding center of the 30*S* subunit, with an orientation that corresponds to the accommodated form in which the acceptor arm is in the PTC. Surprisingly, although full-length aminoacylated $tRNA^{Phe}$ was used in crystallization and its binding was stabilized by paromomycin, the rest of the tRNA was not visible in the density. We believe that this is due to deacylation of $tRNA^{Phe}$ during crystallization, which results in a reduction of affinity for the PTC and a disorder of the acceptor arm. Deacylation also allows the tRNA to bind to the E site.

The ASL of the tRNA, the A-site codon, as well as the 16*S* RNA nucleotides G530, C1054, A1492, and A1493, superimpose perfectly with the structure of ASL^{Phe} in the 30*S* subunit (*13*), confirming the interactions of A1492, A1493, and G530 with the minor groove of the first two codon-anticodon base pairs and the packing of C1054 against the wobble base pair. This also validates studies on decoding using the 30*S* subunit with an mRNA interrupted between the A and P sites (*11*, *13*). There is only one direct contact of the ASL with 23*S* RNA, where A1913 in intersubunit bridge B2a forms a hydrogen bond to the 2′-OH of ribose 37.

The P site. Except during initiation, the tRNA has already been selected by the time it is translocated to the P site, and codon-anticodon interaction is not monitored. Instead, the P site has evolved mainly to hold the tRNA tightly in position to maintain the reading frame and for peptidyl transfer. The entire P-site $tRNA^{fMet}$ was visible in an unbiased difference Fourier map, a portion of which is shown (Fig. 1A). As seen in the 5.5 Å structure (*3*), the P-site tRNA makes extensive contacts with both ribosomal subunits, with 2481 $Å^2$ or 19% of its surface area buried in interactions with the ribosome or mRNA (Fig. 2A). The distinguishing features of initiator tRNA were clearly seen. These include the absence of a Watson-Crick base pair at C1-A72, a purine-pyrimidine base pair, 11-24, and three consecutive GC base pairs in the anticodon stem (*14*, *15*).

Interactions of P-site tRNA with mRNA and the 30*S* subunit. The backbone of the mRNA codon in the P site interacts with C1402, C1403, and U1498 of 16S RNA. In addition, we saw a metal ion that bridged C1401 and G1402 to the codon. G1401 was shown in modification interference experiments to be important for codon-dependent P-site tRNA binding (*16*). The wobble base pair is held in place by stacking interactions from two sides; C1400 stacks with the base pair and G966 against the ribose of C34 (Fig. 2B). Recent mutagenesis on the P site showed that mutation of C1400 to a purine reduces translation at least 12-fold (*17*), presumably because a purine would clash with G966 in the current structure and the distortion required to accommodate it would lower the affinity of P-site tRNA.

Interestingly, A1339 was identified as a critical nucleotide in mutational studies of the 30*S* P site (*17*). A1339 and G1338 of 16*S* RNA form type I and type II A-minor interactions (*18*) with the GC base pairs 30-40 and 29-41 of P-site tRNA (Fig. 3A). These are two of the three consecutive GC pairs in the anticodon stem loop that are characteristic of initiator tRNA and that have been implicated as being important for initiation. Because these interactions are stronger with GC base pairs than AU (*19*), this suggests an explanation for additional stabilization of initiator tRNA compared with other tRNAs. The importance of these minor groove interactions in discrimination of initiator tRNA has been established in recent mutagenesis studies (*20*). However, as the authors point out, it is not entirely clear why three rather than just one or two GC pairs are required; other factors are likely to be important in initiator tRNA selection. Interestingly, A1339 makes a suboptimal A-minor interaction, with the distance between the N1 of A1339 and the 2′-OH of G29 too long for a hydrogen bond (4 Å; marked with an "x" in Fig. 3A). It is possible that during initiation, small conformational changes in the head induced by the binding of initation factor IF3 bring the head into an optimal orientation to inspect the initiator tRNA.

A1339 and G1338 in the head of the 30*S* interacts with the ASL of P-site tRNA on one side, while nucleotide 790 at the tip of the 30*S* platform contacts the ASL on the other side, thus preventing its movement into the E site (Fig. 3B). During translocation of tRNA from the P to the E site, these two elements would have to move apart, presumably by a movement of the head (*4*, *21*). Thus, G1338 and A1339 may not only confer additional stability to initiator tRNAs but may also act as a switch during both initiation and translocation.

Two protein tails from the 30*S* extend into the P site (Fig. 2A) (*22*). Lys^{127} of S9 interacts with the phosphate oxygens of P-site tRNA positions 33 and 34, and the backbone of S13 at residue 118 comes close to the phosphate oxygens of P-site tRNA position 29.

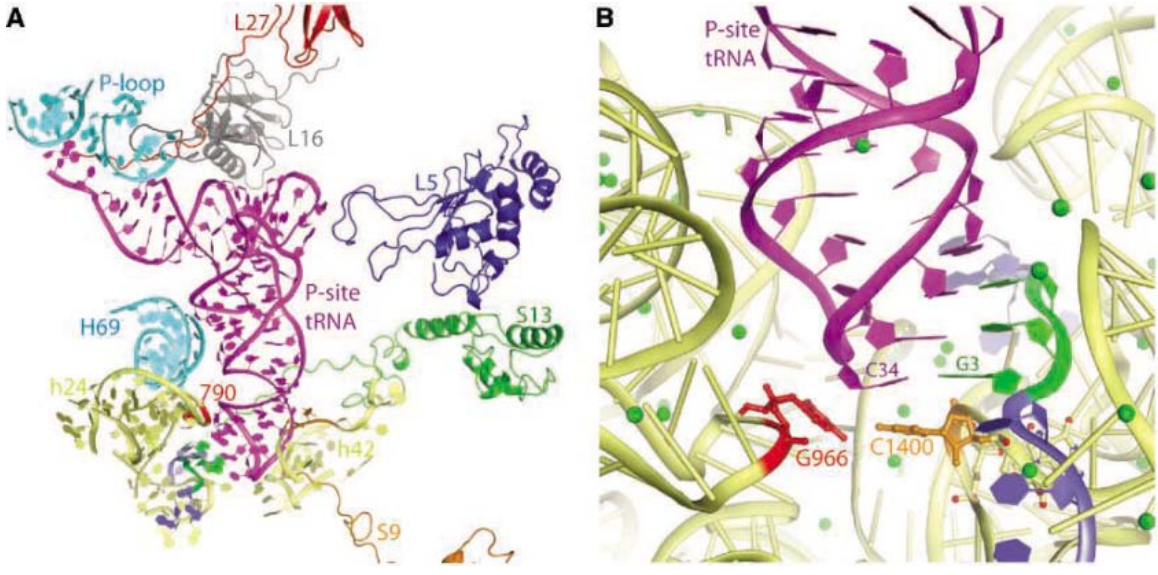

Fig. 2. P-site tRNA interactions with the ribosome. (**A**) Overview of both RNA and protein interactions with P-site tRNA. P-site tRNA interacts with many ribosomal protein tails such as L27, L16, L5, S13, and S9 and 16*S* and 23*S* RNA. 16*S* RNA bases 790 and 1338 and 1339 interact with the anticodon stem, thereby acting as a gate between the P and the E site. (**B**) Interaction of the anticodon loop with mRNA and the 30*S* subunit. The wobble base pair (C34-G3) is held in place from two sides by 16*S* RNA base C1400 that stacks on the wobble base pair and by 16*S* RNA base G966 that stacks against the ribose of C34.

In the structure of the 30*S*, the spur of a symmetry-related molecule interacted with the 3′ end of the 16*S* RNA, mimicking a P-site codon-anticodon interaction (*22*). Because this interaction formed noncanonical pyrimidine-pyrimidine base pairs at all three positions, it was not clear how accurately the structure represented the details of true tRNA-codon interactions. The present structure shows that despite the noncanonical base pairs, the spur in the 30*S* is indeed an excellent mimic of the ASL part of the tRNA. The main differences are that G966 packs optimally against the ribose of anticodon nucleotide 34 and that the upper part of the ASL in the spur of the 30*S* was displaced by 4 Å relative to the P-site tRNA in the 70*S* structure.

The P-site tRNA was distorted relative to the isolated crystal structure of yeast $tRNA^{Phe}$ (fig. S4) (*23*, *24*). A deformation of the anticodon stem was caused by opposing interactions with the head of the 30*S* subunit and H69 of the 50*S* subunit, resulting in an opening up of the major groove around the 26:44 base pair. Interestingly, this means that if constraints on the tRNA were released in the 50*S*, e.g., after peptidyl transfer, relaxation of the deformation would have the effect of driving the tRNA toward the E site (fig. S4).

Interactions at the PTC. The acceptor end of P-site tRNA interacts with the PTC in the 50*S* subunit in a manner similar to that observed for oligonucleotide mimics of tRNA soaked into the *Haloarcula* 50*S* subunit [e.g., (*25*, *26*)], in contrast to the suggestion that the orientation of tRNA in the PTC is determined by remote interactions (*27*). Bases C74 and C75 form Watson-Crick base pairs with G2252 and G2251, while the terminal A76 stacks with 75 and 74 and forms an A-minor interaction with the A2450-C2063 base pair (Fig. 4). The 2′-OH of A76 is in hydrogen-bonding distance of both the N3 and 2′-OH of A2451, showing its importance in stabilizing the conformation of P-site tRNA. The 2′-OH of A76 and nucleotide A2451 are known to be important for peptidyl transferase activity and for substrate stabilization (*28*, *29*).

The PTC itself was in a very similar conformation to those reported in studies of the *Haloarcula* 50*S* with various ligands as well as to the PTC of the *Deinococcus radiodurans* 50*S* subunit (*10*). The A and P loops superimposed closely, with the P loop accommodating an extra nucleotide in the *Haloarcula* structure while closely maintaining the positions of the other bases. Nucleotides known to be mobile in the 50*S* subunit (*28*, *29*), such as A2062 at the entrance to the nascent peptide channel and A2602, which lies between the A and P sites, also appeared to be mobile in our structure, because they had significantly weaker density. The conformation of the PTC was also similar to that of the empty *E. coli* 70*S* structure (*4*). Although concerted differences in the conformation of the PTC between the *E. coli* and *Haloarcula* 50*S* structures were reported (*4*), those differences are small and comparable in magnitude to the difference between the current structure and the *E. coli* structure or to differences between apo- and ligand-bound *Haloarcula* structures (rmsd ~ 0.7 Å).

In *E. coli*, the N-terminal tail of L27 can be cross-linked to A76 of P-site tRNA (*30*), and deletion of as few as the first three residues can significantly reduce peptidyl transferase activity (*31*). These observations suggest that the N terminus of L27 is close to or at the PTC. In the 70*S* structure, L27 has the fold of the crystal structure of the isolated protein (*32*) rather than that reported in the *Deinococcus* 50*S* subunit structure (*10*). It is well defined from residue 10 onward, but very weak density is also visible for residues 1 to 9. The density is consistent with a position of the N terminus close enough to interact with A76 of P-site tRNA, where it could additionally stabilize the P-site substrate and thereby enhance peptidyl transferase activity. This is in contrast to the *Haloarcula* 50*S* structure, in which Asp^{111} of L10e, adjacent to a disordered loop, is 11.5 Å away (*26*). We also find that the closest ordered metal ion is 8.5 Å away from the 3′-OH of A76, in agreement with studies on the *Haloarcula* 50*S* structure reporting the absence of metal ions in the immediate vicinity of the PTC (*26*).

Apart from the ASL and CCA ends, other parts of the P-site tRNA makes extensive interactions with the 50*S* subunit (Fig. 2A). As seen earlier at 5.5 Å (*3*), H69 of 23*S* RNA makes direct minor groove interactions with the D helix (nucleotides 11 and 12) and also with the adjacent nucleotides 24 and 25 in the anticodon stem. At the elbow, protein L5 forms two hydrogen bonds with C56, and L16 is close to G53 but too far to make an interaction in the current structure. Also, we saw five Mg ions coordinated within the tRNA and a sixth bringing together the nonbridging phosphate oxygens of tRNA nucleotides 75 and 76 and 23*S* RNA nucleotide 2602.

The E site. During translation, the ribosomal E site is occupied by deacylated tRNA that has been translocated from the P site. The nature and role of the E site remains controversial. E-site tRNA was postulated to bind both 30*S* and 50*S* subunits and to make interactions with the codon of mRNA (*33*). However, footprinting and other

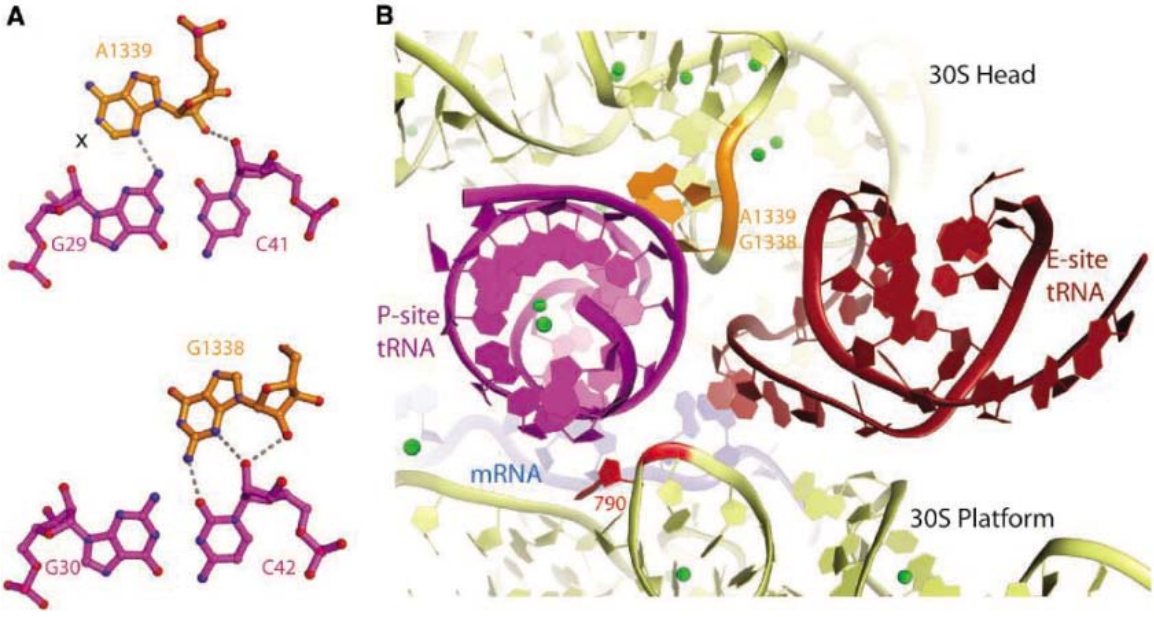

Fig. 3. The gate between P and E site tRNA formed by the head and platform of the 30*S* subunit. (**A**) Top image displays the Type I A-minor interaction of 16*S* A1339 with P-site tRNA base pairs G29-C41, where the x indicates that the distance is too long for the hydrogen bond usually formed in this motif between the N3 of the adenine and the 2′-OH. Bottom image displays the Type II A-minor interaction of 16*S* G1338 with P-site tRNA base pairs G30-C42. (**B**) Key 16*S* nucleotides that act as a gate by preventing P-site tRNA from translocating to the E site (tRNA shown in maroon), as seen looking down at the P-site tRNA from the 50*S* side. This gate is monitored by the 30*S* bases 1338 and 1339 from the head and the base 790 from the tip of the platform. The Mg ions are shown in green and the mRNA in blue in the background.

biochemical data questioned the existence of an E site on the 30*S* (*34*, *35*). The 5.5 Å structure of the *Thermus* ribosome unambiguously established the presence of an E site in both the 30*S* and 50*S* subunits (*3*). However, at that resolution it was unclear to what extent codon-anticodon base pairing occurred.

We saw density for E-site tRNA, and the absence of the insertion at nucleotide 17 that was present in initiator $tRNA^{fMet}$ as well as other features identify it as deacylated $tRNA^{Phe}$. Both the anticodon and CCA ends of tRNA are well defined, whereas some regions around the elbow region are poorly ordered.

The 30*S* E site. We did not observe any codon-anticodon interactions in the E site. In fact, A35, the middle anticodon base, was closer to G693 of 16*S* RNA than to the E-site codon. The tRNA here is noncognate, but it would not be possible to make codon-anticodon base pairs even with a cognate codon unless the mRNA or tRNA were to move significantly relative to their present locations (Fig. 5A). Whereas A and P site tRNAs made extensive interactions with 16*S* RNA, the 30*S* E site is primarily made of proteins S7 and S11, as seen earlier (*3*, *22*), explaining the absence of footprints to 16*S* RNA (*34*).

The 50*S* E site. In the 50*S*, the acceptor end of E-site tRNA interacts with residues at the base of H82, whereas bases at the ends of the T and D loops interact with the L1 stalk, thus stabilizing the stalk in a closed conformation. A number of tRNA mutations and modifications that affect translocation are thought to have altered interactions with the 50*S* E site, presumably during the formation of the P/E hybrid state. The interactions of tRNA with the 50*S* E site were seen in sufficient detail to analyze these data (Fig. 5B).

The base and 2′-OH of A76 have been shown to dramatically affect E-site tRNA binding and translocation (*36*, *37*). The base of A76 intercalates between G2421 and A2422 of 23*S* RNA and makes hydrogen bonds with the universally conserved C2394, as seen recently in the E site of the *Haloarcula* 50*S* subunit, by using a minihelix representing the acceptor arm of tRNA (*38*). The 3′-OH of A76 is surrounded by 23*S* RNA elements, and the site could not accommodate an amino acid on the tRNA, explaining the requirement for deacylated tRNA.

However, the interactions of E-site tRNA in the bacterial 70*S* structure also show striking differences from those in the archaeal *Haloarcula* 50*S* subunit. The orientations of the acceptor arms were different, possibly because the minihelix used in the *Haloarcula* structure lacked the D and T loops that make interactions with the L1 stalk. Moreover, we find that C75 stacks directly on C74, whereas in the archeal structure the base of C75 was splayed out from the tRNA in the opposite direction from A76, where it was stabilized by interactions with L44e (*38*). This protein is not present in bacterial ribosomes, and in any case such a conformation would be precluded by clashes with U2431 and A2432 and by the presence of protein L28 (previously identified as L31).

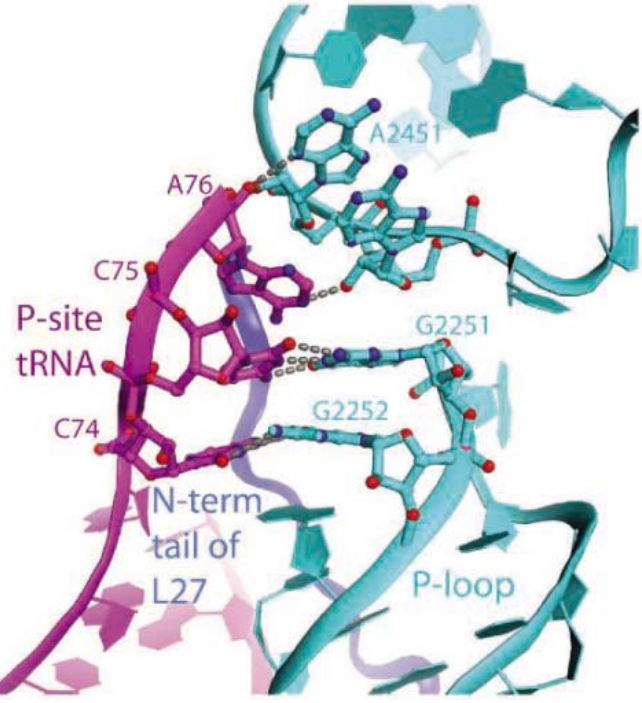

Fig. 4. P-site tRNA interactions at the PTC. The CCA end of P-site tRNA in the PTC interacts with conserved 23*S* RNA bases A2451 and the P loop. Specifically A76 is stacked with both C75 and C74, and its 2′-OH interacts with both the N3 and 2′-OH of A2451. C74 and C75 form Watson-Crick base pairs with P-loop bases G2252 and G2251. The N-terminal tail of L27 (blue) is shown in the background and is known to be close to A76 from biochemical studies.

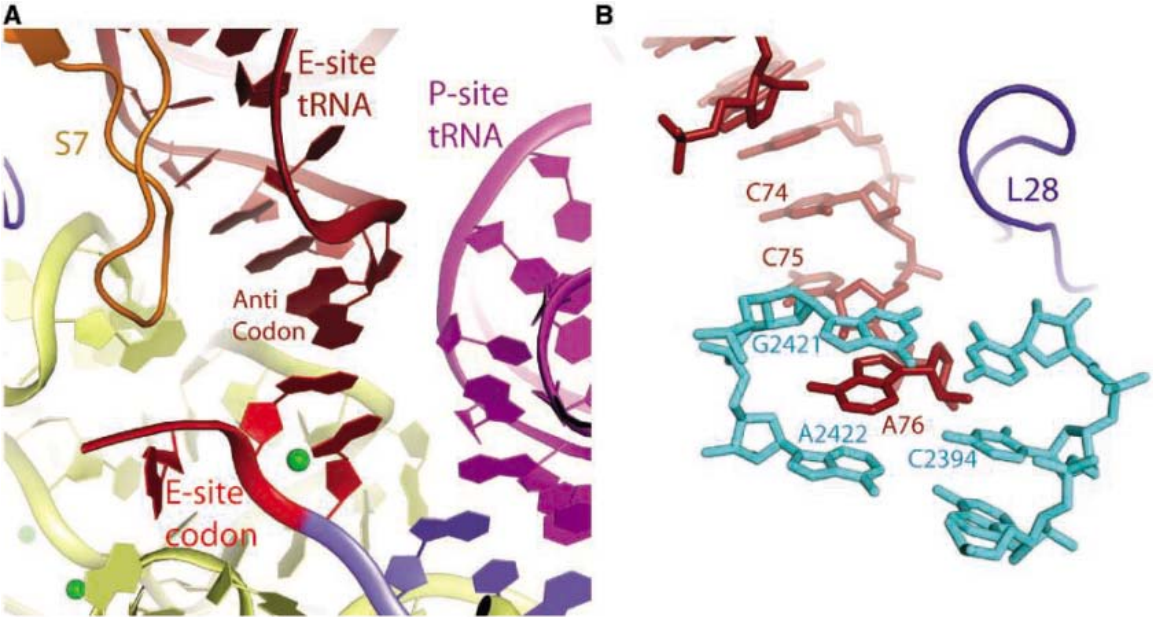

Fig. 5. E-site interactions in the 70*S*. (**A**) The anticodon of a noncognate E-site tRNA does not interact with the E-site codon of mRNA. Mg ions are shown in green. (**B**) A76 at the acceptor end of E-site tRNA intercalates between bases G2421 and A2422 of 23*S* RNA and interacts with the conserved base C2394. C75 is stacked on C74 and is in a markedly different conformation from that previously observed in an archaeal 50*S* subunit (*38*).

The common mode of binding of A76 suggests that the existence of the E site predates the divergence of bacteria from archaea. However, differences in the conformation of C75 and the identities of the proteins present suggest that the E site has subsequently evolved differently in bacteria and other kingdoms.

General features of mRNA. The sharp kink between the A- and P-site codons (*3*) clearly delineates the border between these two sites and presumably is important for defining the reading frame and preventing slippage of the mRNA. In the 70*S* structure, this kink is stabilized by a Mg ion, which makes interactions with the phosphate oxygens of the third P-site nucleotide and the first A-site nucleotide, allowing them to come closer together (Fig. 1C). Interactions of the Mg ion with phosphate oxygens of nucleotides 1401 and 1402 of 16*S* RNA further fix the frame of mRNA with respect to the 30*S* subunit.

Apart from the codons at the tRNA binding sites, we saw two additional nucleotides beyond the E site. The rest of the mRNA appeared disordered, just as in the 5.5 Å 70*S* crystal structure (*3*), although Yusupova *et al*. could see its extended path, including the Shine-Dalgarno interaction at the 5′ end, by using a low resolution difference Fourier map between ribosomes containing and lacking mRNA (*39*).

Overview of proteins. Whereas the 30*S* proteins were little changed from the 30*S* structure of the same species (*2*), all of the 50*S* proteins in the structure had to be rebuilt, some of them ab initio (*8*) (table S3). We report here some significant differences from previous work.

A region of the 50*S* subunit was originally interpreted as corresponding to protein L31 (*10*), and this interpretation was subsequently propagated into low-resolution structures of the 70*S* ribosome as well as that of the higher-resolution *E. coli* ribosome structure (*4*). However, this region of the electron density showed an additional α helix that could be accounted for by the L31 sequence. Moreover, it is a region where cross-links have been observed to ribosomal protein L28 (*40*), whereas no biochemical data connected it to L31. We could satisfactorily build the L28 sequence into the electron density.

At the same time, unexplained density was found for a protein adjacent to protein L5 (fig. S5). We suggest that this is protein L31 on the basis of reports that it formed a cross-linked dimer with L5 (*41*). This location of L31 would also be consistent with the ease with which it dissociates from the ribosome (*42*). Helical density from L31 packs against an α helix of L5 and the intersubunit surface α helix of S13.

We saw no evidence for L36, although the structure of this protein from *Thermus* has been solved in isolation (*43*). Interestingly, the pocket where L36 was seen in the *Deinococcus* 50*S* (*10*) was also empty in the *Haloarcula* 50*S* (*1*). It is possible that the protein was lost during purification, but, given the nature of the pocket and the highly charged nature of L36, we consider this unlikely. An alternative hypothesis is that the protein is not a true ribosomal protein, but this idea would be difficult to reconcile with its presence in the *Deinococcus* 50*S* structure (*10*).

Intersubunit bridges. During translation, ribosomal subunits need to associate during initiation and dissociate during recycling after termination. However, they also need to move relative to each other, especially during translocation (*21*). Because translation is a highly specific and intricate process, the association of ribosomal subunits, as well as the changes in their interaction during relative movement, must be both highly specific as well as dynamic. It has also been known for almost 50 years that ribosomal subunits from all species studied can reversibly associate and dissociate in vitro as a function of Mg concentration, suggesting the universal importance of divalent ions in intersubunit contacts (*44*).

The interactions between subunits occurs through a number of bridges, first seen and named as B1 to B6 in cryo-EM maps of the ribosome (*45*). In particular, the long penultimate h44 of 16*S* RNA extended from the interdomain junction of the 30*S* to the bottom of the subunit and made a number of intersubunit contacts (Fig. 6A). These bridges are essential for subunit association, but some of them also need to be formed and broken during the translation process. The 5.5 Å structure of the ribosome described the molecular components that make up the bridges, subdividing the classification further (*3*). The 3.5 Å structure of the *E. coli* ribosome described many of these interactions in greater detail, especially between components of rRNA. The various bridges differ in character, probably reflecting their nature as static or dynamic contacts. We saw not only the RNA elements but also the side chains of proteins, as well as ions that are involved in the formation of bridges (table S4). Apart from being essential to our understanding of intersubunit interactions, these details help rationalize recent biochemical and genetic data as well as the role of metal ions in subunit association. We describe some examples of bridges: an induced conformational change in a bridge as a result of tRNA binding, a bridge that is entirely mediated by a metal ion, and a bridge in which both metal ions and protein side chains contribute to bridge formation.

In bridge B2a, H69 of 23*S* RNA (*3*, *4*) extends across the interface to interact with h44 of 16*S* RNA. The loop of H69 was disordered in the *Haloarcula* 50*S* (*1*) and formed a compact structure in *Deinococcus* 50*S* (*10*). However, comparison of our structure to the *E. coli* 70*S* structure shows that A1913 of H69 flips to insert into a tight pocket formed by the backbone of h44 and A-site tRNA, forming a hydrogen bond between its N1 to the 2′-OH of A37 of A-site tRNA (Fig. 6B). The base is then oriented toward the bases of A1492 and A1493 that flip out during decoding to interact with tRNA and mRNA (*13*). A Mg ion bridges the ribose O4′ of A1913 of H69 with a phosphate oxygen of position 38 of A-site tRNA, and a second Mg ion bridges the ribose 2′-OH with the nonbridging phosphate oxygens of 16*S* 1493 and 1494. The result of this rather tight interaction in combination with the h44 movement to monitor decoding is that the entire H69 is shifted slightly toward the 30*S* subunit relative to the empty *E. coli* structure. This conformational change may offer one route for signaling correct 30*S* decoding to the 50*S* guanosine triphosphatase center before tRNA accommodation.

B2c is a purely Mg-mediated bridge in which ordered metal ions mediate interactions between the backbones of h24 and h27 of 16*S* RNA and H67 of 23*S* RNA (Fig. 6C and table S4). The structure rationalizes the observation that phosphothiorate substitution at C770 in h24 of 16*S* RNA is not tolerated, presumably because it would inhibit Mg-dependent subunit association via nonbridging phosphate oxygens (*46*). In addition to B2c where they are crucial, metal ions also appear to confer additional stability to B5, B6, and B8 (table S4).

Proteins in the bridges interact with RNA, other proteins, and metal ions. The direct interactions of proteins to RNA in bridges seem to be entirely to the backbone rather than to specific bases. Such interactions may be characteristic of dynamic elements that have to make different contacts in different states of the ribosome. In B5, the only direct interaction between L14 and 16*S* RNA is between Arg^{49} of L14 and a nonbridging phosphate oxygen of nucleotide 1423 of 16*S* (Fig. 6D). Interestingly, a Mg ion is coordinated to the nonbridging phosphate oxygens of 1421 of h44, 1950 and 1951 of H71, and Glu^{54} of L14 (Fig. 6D). Additionally, L14 and L19 also interacted with two ordered Mg ions in B8 (Fig. 6E).

In B6, the minor grooves of h44 and H62 approached each other but left a 6 Å gap as seen in the *E. coli* structure, where it was suggested that there might be a monolayer of water molecules (*4*). We observed one ordered solvent molecule that is coordinated by the backbone of nucleotides 1703 and 1704 of H62 and 1429 and 1430 of h44.

In the *Thermus* 70*S* structure, there appears to be an additional bridge (not present in *E. coli*) involving an interaction of G1442a at the bottom of h44 and a nonbridging phosphate oxygen of 23*S* G2864 of 23*S* RNA that is mediated by an extended loop of L19. This contact might lend some additional stability to the *Thermus* ribosome and is the only example of a protein interaction with an RNA base rather than its backbone.

Conclusions. The structure of the 70*S* ribosome describes the detailed interactions of the

mRNA and tRNA substrates with the ribosome, the interactions between the ribosomal subunits, and the role of metal ions in the structure. The interactions of A-site tRNA in the decoding center and P-site tRNA at the PTC were in good agreement with work done on the 30*S* and 50*S* subunits using oligonucleotide mimics of tRNA. We saw no codon-anticodon interactions with the noncognate tRNA in the E site. The interactions of E-site tRNA with the 50*S* subunit showed both similarities and differences with the previously studied *Haloarcula* E site. The involvement of magnesium ions has long known to be crucial for several aspects of translation, such as subunit association and codon-dependent tRNA binding. We saw metal ions in key positions of critical areas, such the interface between subunits and between the ribosome and tRNA and mRNA. In particular, a magnesium ion stabilized a kink in mRNA at the boundary between the A- and P-site codons, which is of potential importance in preventing slippage during translation. The structure helps to rationalize much detailed biochemical, mutational, genetic, and conservation data and should be useful for the design of future experiments. Moreover, because the A and P sites of the structure are relatively protected from crystal contacts, it is hoped that this crystal form will pave the way for high-resolution structures of functional complexes involving other substrates as well as 50*S* antibiotics that require an occupied P-site substrate.

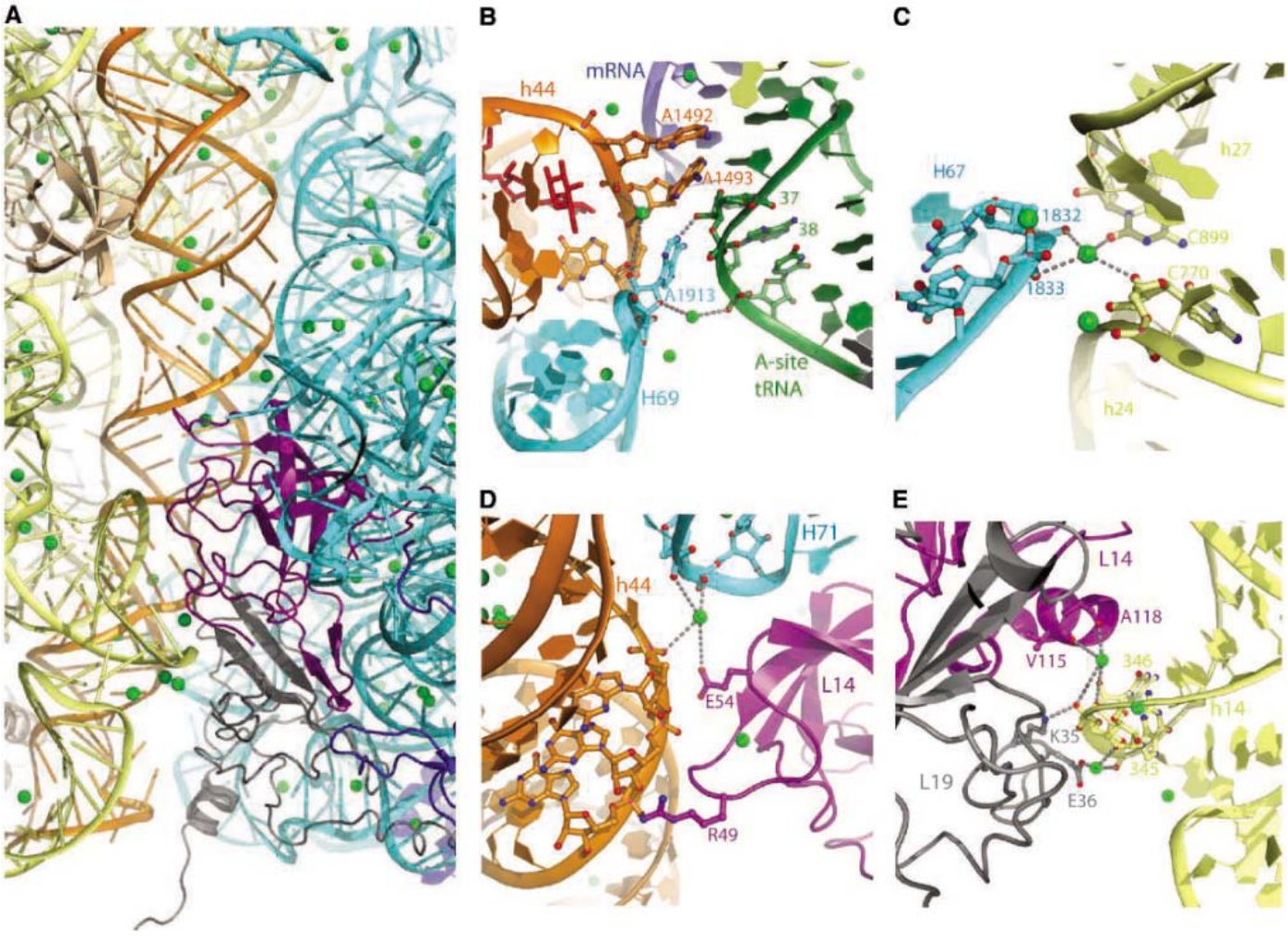

Fig. 6. Examples of the roles of metal ions and proteins in the intersubunit bridges. (**A**) Overview of the extensive intersubunit bridge interactions that 50*S* proteins L19 (gray) and L14 (purple) makes with h44 of 16*S* RNA (orange). L14 is involved in bridge B5 that interacts with 16*S* h44, L19 is involved with bridge B6 that interacts with 16*S* RNA h44, and both L14 and L19 are involved in bridge B8 that interacts with h14. The 23*S* RNA is shown in cyan, 50*S* proteins in blue, 16*S* RNA (except for h44) in yellow, 30*S* proteins in tan, and Mg ions in green. (**B**) An example of an induced change in a bridge on A-site tRNA binding. In bridge B2a, A1913 of H69 of 23*S* RNA flips out of its loop toward 16*S* RNA bases A1492 and A1493 to form a hydrogen bond with A37 of A-site tRNA. Two Mg ions (green) also interact with A1913: One is coordinated between its O4′ and nonbridging phosphate oxygen of U38 of A-site tRNA, and another between its 2′-OH and the nonbridging phosphate oxygens of both 1492 and 1493. Paromomycin is shown in red and mRNA in purple. (**C**) A Mg-mediated bridge. In bridge B2c, a Mg ion (green) mediates the interaction between the RNA backbones of h24 and h27 of 16*S* RNA and H67 of 23*S* RNA. The Mg ion is coordinated between the 2′-OH of 770 and O2 of C899 of 16*S* RNA and the nonbridging phosphate oxygens of 1832 and 1833 of 23*S* RNA. (**D** and **E**) Proteins and ions in bridges. In bridge B5 (D), L14 is involved by either directly interacting with the backbone of 16*S* RNA or via a Mg ion (green) that also contacts the backbone of both 16*S* and 23*S* RNA. Arg^{49} of L14 interacts directly with the backbone of 1423 of 16*S* RNA, and Glu^{54} is coordinated to a Mg ion that in turn interacts with the backbones of nucleotide 1421 of 16*S* RNA and 1950 and 1951 of 23*S* RNA. Bridge B8 (E) consists of interactions of the backbone of h14 of 16*S* RNA with both L14 and L19 via two Mg ions (green). In the center, a Mg ion is coordinated to the backbone oxygens of both Val^{115} and Ala^{118} of L14 and to h14 of 16*S* RNA. Below and to the left, a Mg ion is coordinated between the side chain of Lys^{35} of L19 and h14 of 16*S* RNA. These interactions show the importance of Mg ions in additionally helping to stabilize bridging interactions between the 50*S* and 30*S* subunits.

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48. Crystals were screened at Daresbury Labs, UK, or at European Synchrotron Radiation Facility (ESRF), Grenoble, France. Data were collected at the Swiss Light Source, Paul Scherrer Institut, Villigen, Switzerland, and at ESRF. We thank C. Schulze-Briese and R. Ravelli for help and advice with data collection; W. Kabsch for advice with the data integration program XDS; P. Adams for advice and for modifying the refinement program CNS; D. Gohara for optimally compiling an operating system X version of this modified CNS; P. Emsley for advice with the graphics program COOT; K. Nierhaus and E. Schmitt for gifts of overproducing clones of Phe- and fMet-tRNAs, respectively; and T. M. Schmeing for critical comments. This work was supported by the Medical Research Council (UK), NIH grant GM67624, the Agouron Institute and fellowships from the Wenner-Gren Foundations (M.S.), the American Cancer Society (C.M.D.), European Molecular Biology Organization (F.V.M.), Austrian Academy of Sciences (A.W.), and the Boehringer-Ingelheim Fond (S.P.). Coordinates and structure factors have been deposited with the Protein Data Bank (PDB) with accession codes 2j00 (30S-1), 2j01 (50S-1), 2j02 (30S-2), and 2j03 (50S-2). V.R. holds stock options in and is on the Scientific Advisory Board of Rib-X Pharmaceuticals, a company that develops antibacterial drugs that target the ribosome.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1131127/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 to S4
References

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REPORTS

Tunable Quasi–Two-Dimensional Electron Gases in Oxide Heterostructures

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We report on a large electric-field response of quasi–two-dimensional electron gases generated at interfaces in epitaxial heterostructures grown from insulating oxides. These device structures are characterized by doping layers that are spatially separated from high-mobility quasi–two-dimensional electron gases and therefore present an oxide analog to semiconducting high–electron mobility transistors. By applying a gate voltage, the conductivity of the electron gases can be modulated through a quantum phase transition from an insulating to a metallic state.

Complex oxides show a broad spectrum of intrinsic functionalities, such as ferroelectricity, magnetism, superconductivity, and multiferroic behavior [see (*1*)], which can be used and combined in electronic devices that are based on epitaxially grown heterostructures. Physical properties may arise in such multilayers that are not found in either of their constituents. One example, a conducting quasi–two-dimensional electron gas (q2-DEG) is formed at the interface between the two insulating, dielectric perovskites, $LaAlO_3$ and $SrTiO_3$ (*2*, *3*). The electrons at this interface are highly mobile, with values up to 10^4 cm^2 V^{-1} s^{-1} (4.2 K) having been reported (*2*–*5*), and were found to have densities orders of magnitude higher than the densities of two-dimensional electron gases induced at interfaces in heterostructures based on III-V semiconductors. Exploring whether the q2-DEGs can be applied to fabricate high electron mobility transistor (HEMT)–type field effect devices (*6*), we observed that they can be tuned by altering on the unit cell level the thickness of the $LaAlO_3$ sheets. For $LaAlO_3$ layers that are up to 3 unit cells (uc) thick, highly insulating interfaces are obtained. In field-effect transistor configurations that use such interfaces as drain-source (DS) channels, a phase transition to the conducting state is readily achieved by gate fields. Upon change of their carrier densities with applied electric fields, the q2-DEGs react with a pronounced memory effect.

Previous work revealed the existence of metallic electron gases at $LaTiO_3$-$SrTiO_3$ (*7*) and at $LaAlO_3$-$SrTiO_3$ interfaces (*2*, *3*). Because electron energy loss measurements of $LaTiO_3$-$SrTiO_3$ interfaces showed that the electron gas is confined within a ~2-nm-thick layer (*7*), the gas is described to be quasi–two dimensional. Whereas the $LaTiO_3$-$SrTiO_3$ interface is doped by transfer of electrons from the $LaTiO_3$ to the $SrTiO_3$ (*7*, *8*), for the $LaAlO_3$-$SrTiO_3$ interface two different mechanisms have been reported to generate the gas: In some heterostructures, it was found (*2*, *3*) that the carriers are induced by the polarity discontinuity of the TiO_2-LaO^+ stacking

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sequence at the interface, whereas in other samples, in particular those grown under low oxygen pressure, growth-induced oxygen vacancies in the $SrTiO_3$ were the dominant source of doping (*4*, *5*). We chose a third approach to dope the interface. By using the electric-field effect (*9*, *10*), we reversibly induced the q2-DEG in $LaAlO_3$-$SrTiO_3$ interfaces, providing the possibility to tune the carrier density of the q2-DEG without perturbing the microstructure of the interface.

For field-effect doping, it is desirable to use interfaces that have a low carrier density and, in the extreme case, are even insulating. For $LaAlO_3$-$SrTiO_3$ interfaces, this implies limiting possible doping by the polarity discontinuity as well as by oxygen defects. Although doping by oxygen vacancies is reduced if the $SrTiO_3$ is well oxidized, it is preferable to use ultrathin $LaAlO_3$ layers to avoid possible doping by the polarity discontinuity, which would dope the interface if the flow of electrons from the $LaAlO_3$ into the interface is energetically favorable and kinetically possible. In the polarity discontinuity model, the driving mechanism is given by the polar catastrophe (*11*), which leads to an electric potential, V, across the $LaAlO_3$ that diverges with its thickness, d. The heterostructure can avoid the divergence of V by introducing interface roughness, by moving electrons into the interface, and by adding oxygen vacancies (*11*). Because the energy needed to activate $LaAlO_3$ electrons such that they can move does not depend on d, this naïve consideration suggests that d may have to reach a critical value, d_c, for the interface to become doped and hence conducting.

To analyze the properties of the electron gas, we fabricated and measured field effect samples (Fig. 1) with the techniques described in (*12*). To gain information on the strength of doping by oxygen defects in our samples, we analyzed one d = 6 uc sample by cathodoluminescence. Luminescence was observed with minute intensity only: Under standard measurement conditions (*4*), no indications of oxygen defects were observed.

As the measurements show (Fig. 2A), for the interfaces to be conducting, d has to reach a critical thickness, d_c = 4 uc. All samples with $d \geq$ 4 uc were conducting [sheet conductance (σ_s) $\approx 4 \times 10^{-3}$ ohm^{-1} (at 4.2 K) and $\sigma_s \approx 2 \times 10^{-5}$ ohm^{-1} (at 300 K)]; all samples with $d < d_c$, insulating ($\sigma_s < 2 \times 10^{-10}$ ohm^{-1} at all temperatures T). The observation of a d_c of 4 uc is consistent with the observation that the conductivity of $SrTiO_3$-$LaAlO_3$-$SrTiO_3$ heterostructures is reduced if their p and n interfaces are spaced by less than 6 uc (*13*).

Control measurements were performed on samples that were patterned to have conducting interfaces with 5-uc $LaAlO_3$ layers in the areas in which the contacts were placed and subcritical, 2-uc- or 3-uc-thick bridges connecting these areas. These samples are insulating and thereby provide evidence that the critical-thickness phenomenon is not simply caused by an effect that is generated by the contact between the Au and the q2-DEG. Further reference studies on samples contacted without Ar ion–etched holes proved that the conducting layer is not located at the surface of the $LaAlO_3$. The conductivity also does not occur in bulk $SrTiO_3$, as was reported (*14*) for samples grown under less oxidizing conditions. To test for bulk conduction, we removed the surface layer of a conducting sample by polishing. The remaining substrate was highly insulating.

According to Hall measurements done on the conducting samples, their carriers are negatively charged with mobilities of ~1200 cm^2 V^{-1} s^{-1} and ~6 cm^2 V^{-1} s^{-1} at 4.2 K and 300 K, respectively, and densities $n \approx 10^{13}$ cm^{-2} at all T (Fig. 2B). These mobilities are high but lower than the best values reported in literature (*2*–*5*), probably because of the growth conditions used, which were selected to obtain interfaces with low carrier density.

Which information does the steplike dependence of the interface conductance on d provide for the doping mechanisms present in these samples? The d dependence of the interface conductance can only be accounted for by doping from growth-induced oxygen defects, if during sample fabrication oxygen can diffuse well through 3-uc-thick layers but not through 4-uc-thick ones. For this case, one has to expect that $d \geq$ 4 uc samples can be turned into insulators, too, if the diffusion of oxygen through their $LaAlO_3$ layers is enhanced. To test this prediction, we annealed a d = 4 uc sample for 7 days at 400°C in 20 bar of O_2. This oxidation step did not result in an insulating interface but reduced the conductance by a factor of 5 (at all T). It therefore has to be concluded that the d dependence of the interface conductance agrees with the behavior predicted for doping by the polarity discontinuity, although additional doping by oxygen vacancies might still be present.

The insulating samples are well suited to generate and control a q2-DEG by the electric field effect. Electric fields were induced across the $SrTiO_3$ or across the $LaAlO_3$ by applying gate voltages, $V_{G,b}$, to backside contacts of the $SrTiO_3$ or voltages, $V_{G,f}$, to small test contacts silver-

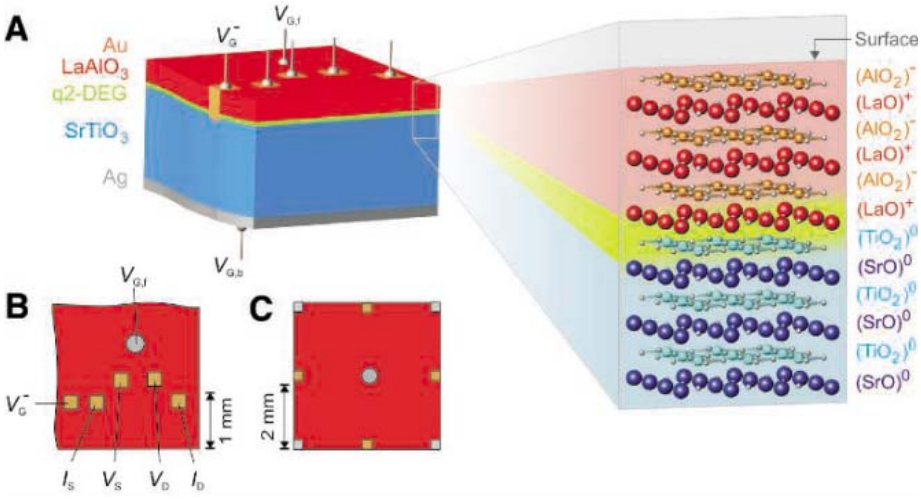

Fig. 1. Sketch of the samples and the contact configurations [the left side of the sample sketch in (**A**) is a cross-sectional cut]. The $SrTiO_3$ substrates were 1 mm thick. Current-voltage and resistance-temperature measurements were done with the configuration shown in (**B**); Hall measurements, with the van der Pauw configuration of (**C**) using either the contact configuration shown in gold or in silver. The charges listed in the lattice sketch represent the unrelaxed charge distribution.

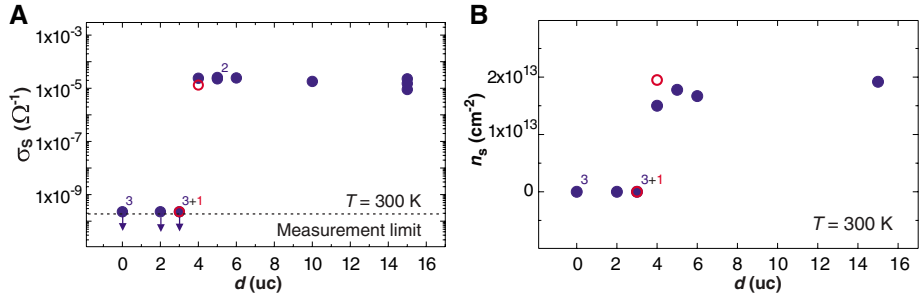

Fig. 2. Influence of $LaAlO_3$ thickness on the electronic properties of the $LaAlO_3$-$SrTiO_3$ interfaces. (**A**) Sheet conductance and (**B**) carrier density of the heterostructures plotted as a function of the number of their $LaAlO_3$ unit cells. The data shown in blue and red are those of samples grown at 770°C and 815°C, respectively. The data were taken at 300 K. The numbers next to the data points indicate the number of samples with values that are indistinguishable in this plot.

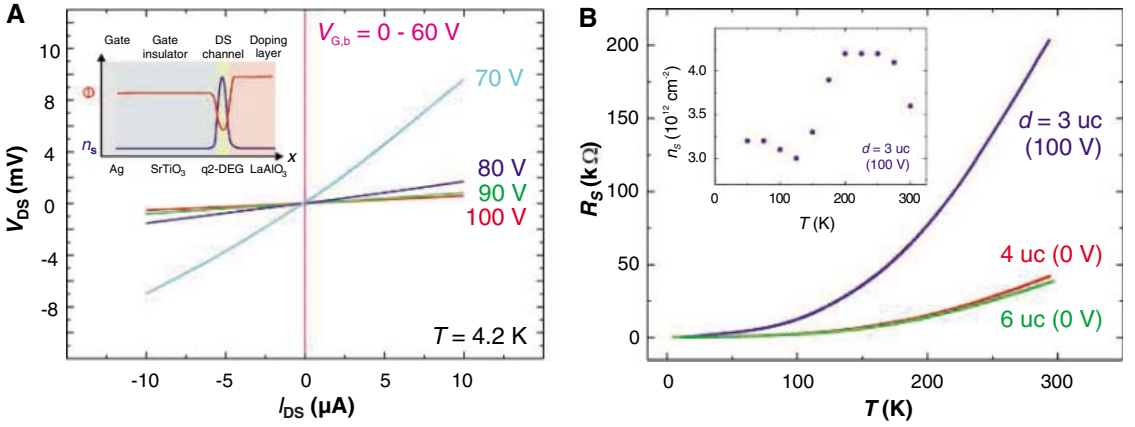

Fig. 3. Transport characteristics of the q2-DEG as a function of applied gate field. (**A**) Voltage-current characteristics of a heterostructure with 3 uc of $LaAlO_3$, measured at 4.2 K with various voltages applied to its back gate. The sample shows a large field-effect response with a conductance change of seven orders of magnitude. The curve taken at $V_{G,b} = 0$ was measured in a two-point configuration, because the resistance was too high to produce a steady voltage between the two voltage contacts of the four-point setup. Several curves display a curvature and an asymmetry, as we observed frequently for high-resistance samples grown on $SrTiO_3$ (*26*). It is attributed to non-ideal and non-identical contacts and to the non-ideal four-point configuration. (Inset) Illustration of the device configuration. Controlled by the spatial dependence $\Phi(x)$ of the electron potential, the q2-DEG is generated at the $SrTiO_3$-$LaAlO_3$ interface, forming the DS channel. (**B**) Sheet resistance measured as a function of temperature for two samples with $d \geq d_c$ at $V_G = 0$ and for one sample with $d = 3$ uc $< d_c$, in which the q2-DEG was induced by $V_{G,b} = 100$ V. The DS current was 1 μA. (Inset) The carrier density of the latter sample (Hall measurements).

painted onto the $LaAlO_3$ (Fig. 1). Also for the gate contacts on 3-uc-thick $LaAlO_3$ layers, the gate resistances were very large (>2 Megohm cm^{-2}), suggesting pinhole-poor (or even pinhole-free) $LaAlO_3$ layers. Independent of which gate electrode was used, pronounced field effects were observed, as anticipated from the weak electrostatic screening of the ultrathin q2-DEG. As illustrated (Fig. 3A), by applying a positive gate voltage, we induced electron gases with sheet conductances as high as $\sigma_s = 5 \times 10^{-3}$ ohm^{-1} (4.2 K) at the interfaces of the 3-uc samples (5×10^{-6} ohm^{-1} at 300 K). The gate field generated metal-insulator transitions with conductance changes exceeding seven orders of magnitude. Driven by the gate voltage and not by thermal activation, this metal-insulator transition is a quantum-phase transition. The sign of the field effect is in accordance with the formation of *n*-doped electron systems.

In samples with $d = 2$ uc, the insulating phase could be barely switched into the conducting one, which shows that the $LaAlO_3$ layers with the subcritical thickness $d = 3$ uc still support the formation of the q2-DEG induced by the electric field. Also in samples with $d \geq 4$ uc, which are already conducting at $V_G = 0$, the conductances were modulated by V_G. However, in these samples a metal-insulator transition could not be induced. According to Hall measurements, the carrier densities of the field-induced q2-DEGs are $\approx 3 \times 10^{12}$ to 4×10^{12} cm^{-2} (Fig. 3); the conductances, mobilities, and $R_S(T)$ dependencies of the field-induced q2-DEGs are reminiscent of the properties of the electron gases that are present in samples with $d \geq d_c$.

The carriers in the DS channels have a high mobility at low *T*. Although not incorporating a spacer layer, these field effect devices are an analog to HEMTs built from conventional semiconductors, because the channel carriers are doped from the $LaAlO_3$ layers, which are spatially separated from the DS channels (Fig. 3A, inset). These

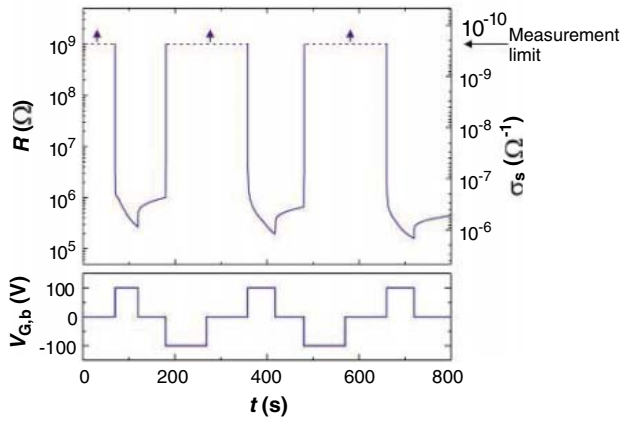

Fig. 4. Memory behavior of the q2-DEG. (**A**) Sheet resistance measured at 300 K and (**B**) applied backgate voltage, both plotted as a function of time for a sample with $d = 3$ uc. By applying the gate voltage pulses, we could reversibly switch the sheet conductance between $\sim 1 \times 10^{-6}$ ohm^{-1} and an unmeasurable value $< 2 \times 10^{-10}$ ohm^{-1}. The data were measured in four-point configurations.

heterostructures apply the concept of modulation doping commonly used in semiconducting heterostructures to oxides (*15*). Yet, there are differences between both systems: On the one hand, the mobilities in the semiconducting interfaces are three orders of magnitude larger than those in the current oxide interfaces, and the carrier densities of the q2-DEG in the oxides exceed those of their semiconductor counterparts by orders of magnitude. Because of the proportionally larger fill factors of the oxide q2-DEGs ($\upsilon = n\Phi_0/B$, where Φ_0 is the magnetic flux quantum and *B* is the applied magnetic flux density), quantum Hall effect–induced resistance oscillations are expected to occur at such oxide interfaces at very large field strengths only. On the other hand, modulation doping of oxides is by principle not restricted to titanates and can be done using many compounds with strongly correlated electron systems. Interactions between the q2-DEG and the correlated electron systems of the bulk may therefore create electronic systems with unique properties.

The field effects shown by the samples are enormous. Field-effect devices that use the $SrTiO_3$ surface as a DS channel have been fabricated with fine characteristics (*16*–*18*), yet the on/off ratios exceed those of these earlier devices by orders of magnitude. There are several reasons: The q2-DEG structures used are close to optimal to achieve large electric-field effects, because the DS channels consist of ultrathin, and therefore weakly screening, electron gases in which a metal-insulator phase transition is induced by the field. In addition, the gate insulator $SrTiO_3$ has a very large electric permittivity (*19*–*21*), which enhances the field response. Furthermore, because there is no conducting DS channel when the sample is insulating, the gate field lines end at the drain and source contacts. Once the gate voltage exceeds a threshold value, the DS channel grows in a bootstrapping mode. This nonlinear process is expected to contribute to the abrupt change of resistance with gate field (Fig. 3).

At 300 K, the electric field–induced q2-DEG was found to display unusual behavior, in particular in samples that over minutes or hours had been subjected to a large $V_{G,b}$ (>70 V). Varying $V_{G,b}$ causes the conductance to react rapidly by a small amount, followed by a larger but very slow change. If the gate is switched, for instance, from $V_{G,b} > 70$ V to $V_{G,b} = 0$ V, the samples with $d < d_c$ retain for hours a high and only slowly diminishing conductance. By application

of a negative $V_{G,b}$, we instantaneously switched off this conductance (Fig. 4). The effect was more pronounced in the samples grown at lower temperature (770°C). Although this memory behavior is reminiscent of ferroelectric field-effect devices (*10*, *22*), the effect is not known for field-effect devices that use dielectric gate insulators.

Within the available V_G window (–2 V < $V_{G,f}$ < 4 V) where V_G also drops along the channel, the memory effect was not found for fields applied via the $LaAlO_3$. Because no mechanism is known that would yield a memory behavior for an isolated q2-DEG, one has to conclude that the $SrTiO_3$ plays a significant role. Indeed, it has been proposed that charge excitations in $SrTiO_3$ can strongly influence the properties of the q2-DEG (*23*). The long, temperature-dependent time constants suggest that creation and motion of defect states, such as oxygen defects, are controlling the dynamics of the effect. We propose that the gate field and also the channel charge give rise to a sheet of positively charged defects or trapping states in the $SrTiO_3$. In this model, the strong electric field of the resulting q2-DEG–defect sheet dipole layer stabilizes the q2-DEG as well as the defect sheet, so that a nominally bistable configuration is obtained. Indeed, being a quantum paraelectric in which the phase transition to the ferroelectric state is suppressed by quantum fluctuations only, already undisturbed bulk $SrTiO_3$ is almost ferroelectric (*24*, *25*).

Operating at 300 K with a large on/off ratio, these field effects are of potential interest for device applications. Although it remains to be clarified whether the device parameters, stability, and integratibility will allow for implementation in practical devices, these heterostructures prove that the coupling of the q2-DEGs to gate fields and to the electronic degrees of freedom of neighboring, complex oxides opens new routes for the design of devices in oxide electronics.

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Ultrafast Vibrational Dynamics at Water Interfaces

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Time-resolved sum-frequency vibrational spectroscopy permits the study of hitherto neglected ultrafast vibrational dynamics of neat water interfaces. Measurements on interfacial bonded OH stretch modes revealed relaxation behavior on sub-picosecond time scales in close resemblance to that of bulk water. Vibrational excitation is followed by spectral diffusion, vibrational relaxation, and thermalization in the hydrogen-bonding network. Dephasing of the excitation occurs in ≤100 femtoseconds. Population relaxation of the dangling OH stretch was found to have a time constant of 1.3 picoseconds, the same as that for excitation transfer between hydrogen-bonded and unbonded OH stretches of water molecules surrounded by acetone.

Water interfaces play an essential role in many physical, chemical, and biological processes. Wetting, corrosion, membrane function, environmental pollution, and soil weathering are just a few familiar examples (*1*, *2*). The properties of both bulk and surfaces of water are governed to a large extent by the network of hydrogen bonds linking the water molecules (*3*). The network is highly dynamic, and recent time-resolved vibrational spectroscopic studies on the sub-picosecond time scale have generated a host of valuable information on hydrogen (H) bonding in bulk water. The focus has been on the OH stretch modes, because they are strongly correlated with H bonding (*3*) and their dynamics over tens to hundreds of femtoseconds reflect directly the dynamics of excitation and relaxation in the H-bonding network (*4*–*6*).

However, hardly any reports on dynamic studies of water interfaces exist in the literature, not because of lack of interest but because of experimental difficulties. Given that water surfaces have a quite different structure than that of the bulk, one might suspect that their vibrational dynamics would be very different as well. Another possibility would be domination of the dynamics in both cases by the H-bonding network, resulting in general similarity of surface and bulk dynamics. Would the reduction of neighbor interactions due to termination of the H-bonding network at the surface make the surface dynamics slower? Alternatively, would the more ordered surface structure lead to faster surface dynamics, as suggested by the observed ultrafast dynamics in isotopically diluted bulk ice compared with bulk water (*7*)?

In recent years, surface-specific sum-frequency vibrational spectroscopy (SFVS) has proven well suited to probing static vibrational spectra of water interfaces. The technique has helped to identify icelike, liquidlike, and dangling OH structures in the terminated H-bonding network of the water interfacial layer (*8*–*12*). In order to probe surface vibrational dynamics of water, time-dependent SFVS is also the technique of choice, although the signal is expected to be much weaker compared with those of the bulk studies. We report here a successful ultrafast dynamics study on neat water interfaces using SFVS. We conducted a surface spectral hole burning experiment in which a femtosecond infrared (IR) pump pulse first created a hole in the spectrum of OH stretches and subsequently time-delayed SFVS probed the evolution of the hole. We observed a recovery time of ~1.3 ps for the dangling OH stretch mode, substantially slower than the OH dynamics in bulk water. In the bonded OH region, however, the observed dy-

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namics were fairly similar to those in bulk water; the recovery underwent changes on similar time scales as in the bulk and dictated by spectral diffusion, vibrational relaxation, and lastly thermalization in the H-bonding network (*4*, *13*, *14*). The results suggest that the dynamics are governed more by H bonding between molecules than by the configuration of the H-bonding network.

The basic principle of surface-specific SFVS can be found elsewhere (*15*). The SF signal reflected from an interface is proportional to $|\hat{e}_{SF}\cdot\overleftrightarrow{\chi}_S^{(2)}(\omega_{SF}=\omega_{vis}+\omega_{IR}):\hat{e}_{vis}\hat{e}_{IR}|^2$, where $\overleftrightarrow{\chi}_S^{(2)}(\omega_{SF}=\omega_{vis}+\omega_{IR})$ is the surface nonlinear susceptibility tensor and $\hat{e}_i$ is the unit polarization vector of the field at ω_i. In the case of spectral hole burning, $\overleftrightarrow{\chi}_S^{(2)}$ is equal to $\overleftrightarrow{\chi}_{S0}^{(2)}$ initially but becomes time-dependent after the pump pulse excitation. Information about surface structural dynamics is contained in the time variation of $\overleftrightarrow{\chi}_S^{(2)}$.

The experiment was carried out with a 1-kHz Ti:sapphire laser pumping two $KTiOPO_4$-based optical parametric amplifier systems to produce tunable pump and probe IR pulses of 130 and 160 fs durations, respectively, and 70 to 85 cm^{-1} spectral width. The pump energy was typically ~10 μJ per pulse and the probe, ~5 μJ per pulse. Time-resolved SFVS probing was achieved by overlapping the IR probe pulse with the Ti:sapphire fundamental laser pulse on samples at different time delays after the IR pump pulse. The spot sizes of the IR pump, IR probe, and 800-nm laser pulses at the sample were ~300, ~400, and ~150 μm, respectively. The SF signal was collected in the reflected direction. Two water interfaces at room temperature were investigated, one with silica to study dynamics of bonded OH modes and the other with octadecyltrichlorosilane (OTS)-coated silica to study dynamics of the dangling OH mode. Deionized distilled water at pH = 5.7 was used in all the experiments. In both cases, a silica prism was used as the substrate so that the total-internal-reflection geometry could be used to probe the interfaces. The finite differences in incident angles of the beams resulted in a lengthening of the third-order correlation width between the IR pump and the SF probe pulses to ~170 fs. The SF output, the 800 nm pulse, the IR probe, and the IR pump were S-, S-, P-, and P-polarized, respectively (S and P refer, respectively, to polarization perpendicular and parallel to the plane of incidence).

We consider first the measurement of the dangling OH mode at 3680 cm^{-1} (Fig. 1B), which is characteristic of a hydrophobic water interface (*8*). Because the peak was much narrower than the pump bandwidth, no spectral hole burning was observable. The SF signal simply probed the population recovery of the mode after pump excitation, which occurred with a single exponential time constant of 1.3 ± 0.1 ps (Fig. 1A). The dephasing time of the mode is ~300 fs, as judged from the peak width in the static spectrum of Fig. 1B.

Spectral hole burning was observed in the bonded OH stretch region of the water-silica interface (Fig. 2, A and B). Initially, the spectral holes were appreciably broader than the pump bandwidth and changed with time, indicating the onset of spectral diffusion or energy transfer between OH stretch modes before relaxation (*6*, *16*, *17*). At longer time delays, the signal recovery overshot at the blue side and undershot at the red side of the broad OH stretch band. To probe the recovery more explicitly, we monitored the SF signal at more closely spaced time delays with various probe frequencies after pumping either band (Fig. 3, A and B). At each probe frequency (except 2900 cm^{-1} with pump at 3200 cm^{-1}), the signal first decreased because of hole burning and rapid spectral diffusion (and an opposite contribution due to transitions from the transiently populated $v = 1$ excited state to the $v = 2$ state) and then recovered toward a quasi–steady state value that lasted over 50 ps. This value is appreciably higher than the initial equilibrium value on the blue side of the OH stretch band and lower on the red side. As in bulk water dynamics, the signal recovery can be fit to a biexponential decay, with time constants of 300 and 700 fs [compared to population relaxation of ~200 fs (*4*, *13*, *14*) followed by thermalization with a time scale of ~550 to 800 fs (*13*, *14*, *18*) in the bulk case] and pre-exponential coefficients that vary with pump and probe frequencies.

The homogeneous dephasing time, T_2, of the bonded OH stretches can be deduced from the spectral width of the initial hole profile. However, because of the finite pulse duration of the pump and probe, the earliest time we could probe the spectral hole was at a probe delay of

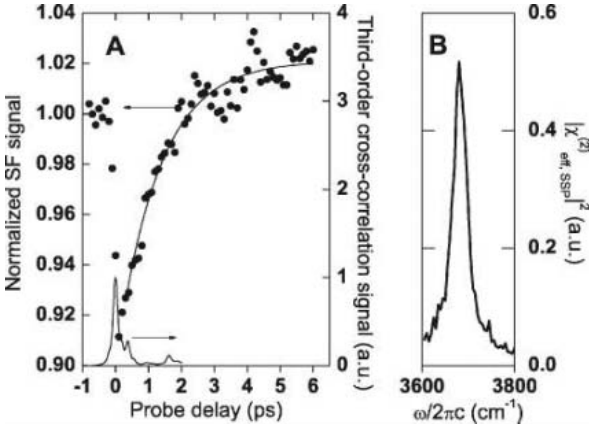

Fig. 1. (**A**) Time-resolved SF probing of the population relaxation of the dangling OH mode at the water–OTS–fused-silica interface. The solid curve is a single-exponential fit to the data, yielding a relaxation time of 1.3 ± 0.1 ps. Also shown is the third-order cross-correlation between the IR pump and the SF probe pulses (arrows). (**B**) The SF spectrum of the dangling OH mode obtained with a narrow-band picosecond laser-optical parametric amplifier system. a.u., arbitrary units.

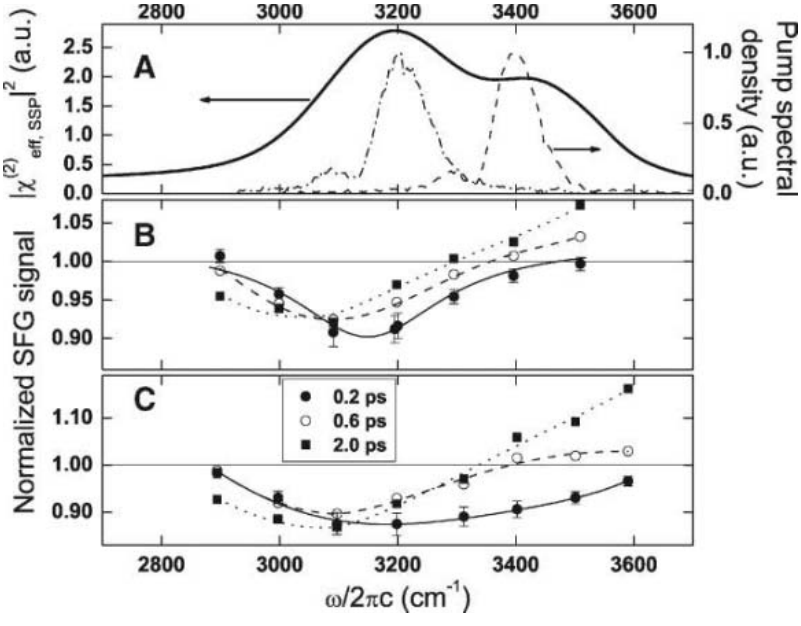

Fig. 2. Spectral hole burning at water–fused-silica interface. (**A**) The SF vibrational spectrum of the interface (solid curve) obtained with a narrow-band picosecond laser-optical parametric amplifier system and the spectral profiles of the pump pulse at 3200 cm^{-1} (dash dotted curve) and 3400 cm^{-1} (dashed curve). (**B** and **C**) The spectral holes induced in the SF vibrational spectrum by the pump at 3200 cm^{-1} and 3400 cm^{-1}, respectively, at three pump-probe delay times. The SF signal at each probe frequency is normalized by the SF signal before the IR pump. The solid curve in (B) is a Lorentzian fit to the data points for a pump-probe delay of 200 fs. The curves on the other data sets in (B) and (C) are guides to the eye. Error bars indicate the standard error of the mean.

~200 fs. The observed spectral profile created by the pump at 3200 cm^{-1} (Fig. 2B) could be fit by a Lorentzian with a width corresponding to a T_2 of ~100 fs. The spectral hole created by the pump at 3400 cm^{-1} was appreciably broader, with its peak red-shifted from the pump frequency, and supported an effective T_2 of ~50 fs.

In a separate experiment, we attempted a photon-echo measurement to obtain T_2. The IR photon echo originating from successive pulse excitations of OH stretch modes at the water interface, if strong enough, could be detected by up-conversion via surface-specific SF generation (*19*). In the simple model for this echo process (*19*), two pulsed IR fields, E_1 and E_2, separated by a delay, Δt, produce a polarization echo at 2 Δt that is up-converted to SF with a relative signal intensity of $SF_{echo}/SF_1 = \sin^4(\theta/2)\exp(-4\Delta t/T_2)$, with SF_1 being the SF signal generated by up-conversion of the vibrational polarization created by E_1 alone. Here, θ is defined as $\frac{\mu_{10}}{\hbar}\int E_2(t)dt$, with μ_{10} being the transition dipole moment that describes E_2-induced vibrational excitation, and can be deduced from measurement of SF_1 and $SF_{1,pump}$, which is the pump-probe SF signal from up-conversion of E_1 immediately after the E_2 excitation: $\sin^2\theta = (SF_1 - SF_{1,pump})/SF_1$. We found that, even with a pump fluence sufficient to produce $\sin^2\theta \sim 0.35$ at 3200 cm^{-1}, the echo signal at $\Delta t = 150$ fs was only $SF_{echo}/SF_1 \sim 2 \times 10^{-5}$. This result allowed an estimate of $T_2 \leq 100$ fs.

To explain our results, we note first that there are many similarities between our observations and those of bulk water (*4*, *13*, *14*). In the bulk, the ultrafast dynamics occur in several stages after excitation: During the first 50 fs, the spectrum shows bleaching (decreased absorption) around the pump frequency and enhanced absorption at the red end of the OH stretch band (*4*, *13*, *14*). These features result from vibrational excitation of molecules from the $v = 0$ to $v = 1$ states of the stretch modes and a rapid redistribution of the excitation energy in stretch modes of neighboring molecules through intermolecular interactions. Next, bleach recovery and accompanying decay of the enhanced absorption occur over ~200 fs, due to population relaxation of the $v = 1$ state. Lastly, on the time scale of 550 to 800 fs, a spectrum emerges at a somewhat higher equilibrium temperature than that preceding the pump because of heating from relaxation of the deposited excitation energy (*4*, *13*). Dephasing of the excitation appears to occur in ~50 fs (*4*). Similar dynamics appear in dilute HDO in H_2O or D_2O except for the stretched time scales due to dilution of the probed vibrational modes (*20*, *21*). Recently, in a study of dynamics of individual water molecules surrounded by a few acetone molecules in CCl_4, Gilijamse *et al*. found that transfer of excitation from the unbonded to bonded OH stretch modes of confined water molecules occurs through the transient breaking of hydrogen bonds with a time constant of 1.3 ± 0.2 ps (*22*).

We can explain the observed surface dynamics of water interfaces along similar lines, in terms of relaxation in the interfacial H-bonding network. The measured population relaxation time of 1.3 ± 0.1 ps for the dangling OH bond matches remarkably well with that of the water molecules surrounded by acetone observed by Gilijamse *et al*., suggesting that the underlying mechanism for relaxation is the same (*22*). Next, we follow the model of Bakker and co-workers and others to discuss the dynamics initiated by excitation of bonded OH stretches (*14*, *21*). We assume that, after a pump-probe delay of $t \sim 100$ fs, spectral diffusion is nearly complete. The dominant mechanism for spectral diffusion among the bonded OH modes is near-resonant vibrational energy transfer, which is known to occur on a sub-100-fs time scale in bulk pure water (*4*, *16*). The broadened and red-shifted spectral hole was a consequence of redistribution of excitations in the available stretch modes and decreased absorption from $v = 0$ to $v = 1$ and enhanced absorption from $v = 1$ to $v = 2$ of the excited modes. By $t \sim 2$ ps, the quasi-equilibrium spectrum is blue-shifted because of heating of the system (*23*). As in the bulk case, we can describe the relaxation dynamics in terms of population relaxation of the $v = 1$ excited state and thermalization toward quasi-equilibrium in the H-bonding network. All the experimental decay traces at delays ≥ 100 fs in Fig. 3, A and B, can be fit reasonably well by the approximate expression derived from Bakker's model (*13*, *14*):

$$S(t) = 1 - (1 - S_0)e^{-(t-100)T_v} + \Delta S[1 - e^{-(t-100)/T_t}] \quad (1)$$

where S_0 is the SF signal at $t = 100$ fs and $1 + \Delta S$ is the signal at quasi-equilibrium ($t \sim 2$ ps), with the signal at equilibrium ($t \ll 0$) set to be 1. The time constants for vibrational relaxation and thermalization deduced from the fits for all traces are $T_v \sim 300$ fs and $T_t \sim 700$ fs. For probing at 3300 cm^{-1} (with pump at either 3200 or 3400 cm^{-1}), $1 - S_0$ is much greater than $|\Delta S|$, and the decay is dominated by vibrational relaxation. With the probe at 2900 and 3600 cm^{-1} (pumped at 3400 cm^{-1}) as well as at 2900 and 3500 cm^{-1} (pumped at 3200 cm^{-1}), we find $1 - S_0 \ll |\Delta S|$ and thermalization dominates.

The great similarities between bonded OH dynamics in bulk and interfacial water suggest that the differences in the structures of the H-bonding networks in the two cases, such as more ordering and termination of the network at interfaces, are not very important on ultrafast time scales. Although SFVS probes only the first one or two water monolayers at an interface (*10*), pumping of the vibrational excitations occurs in an interfacial layer thickness on the order of a reduced wavelength. Rapid excitation transfer and thermalization in the H-bonding network should force the dynamic behavior of interfacial monolayers to be dictated by that of the pumped interfacial layer.

We have not included the possible effect of orientational dynamics of water molecules in our data analysis because the more ordered interfacial water structure, evidenced from their SF spectra (*10*), high surface tension, and low dielectric constant (*1*, *3*), tends to severely restrict the molecular orientational motion. The surface

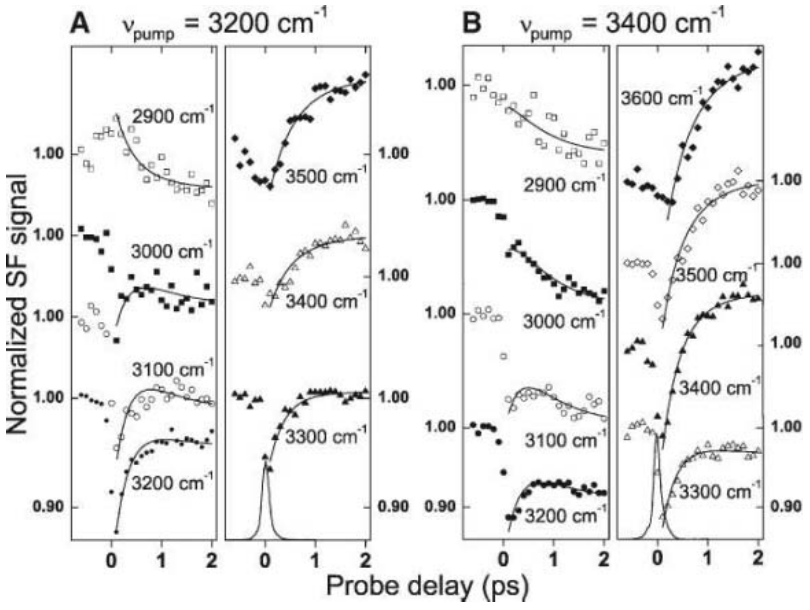

Fig. 3. Time-resolved SF probing of the spectral holes induced by the pump pulse at (**A**) 3200 cm^{-1} and (**B**) 3400 cm^{-1} and described in Fig. 2, B and C. The data show the SF signal, normalized against that in the absence of pump, as a function of pump-probe delay at different probe frequencies. The solid curves are biexponential fits to the data using Eq. 1. The traces for different probe frequencies are displaced vertically for clarity. The solid pulses at the bottom of the right-hand graphs in (A) and (B) describe the third-order cross-correlation traces of the IR pump and the SF probe pulses (linear vertical scale not shown).

orientational dynamics are expected to occur on a time scale significantly longer than the bulk molecular reorientational time of 1.5 ps (*21*). For water molecules surrounded by acetone (*22*) and in reverse micelles (*24*, *25*), the reorientational time was found to be at least 6 ps (*26*).

Our results appear to reflect the fundamental dynamics of the OH stretch modes and associated H bonds connecting neighbors and, as such, are insensitive to the detailed configuration of the H-bonding network. They support the general proposition that vibrational excitation and relaxation of H-bonding molecules in condensed media are governed by vibrational relaxation and energy transfer through H bonds. The experimental technique successfully demonstrated here should also open broad opportunities for ultrafast surface dynamic studies of liquids in general.

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23. The thermalized SF spectrum after long pump-probe delay appears blue-shifted with respect to the unpumped SF spectrum, but the blue shift is somewhat different from that observed in the IR transient absorption spectrum of bulk water (*13*, *14*). This is because the SF spectrum of the water-silica interface has a different temperature dependence (*9*) than that of the IR absorption of bulk water.
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26. Resonant energy transfer among OH stretch modes may also result in temporal variation of the orientational distribution of interfacial molecular excitations. Such orientational dynamics are not important for the bonded OH stretch region in our study because it is more or less complete in 100 fs. For the dangling OH stretch, resonant energy transfer between dangling OH bonds is expected to occur on a time scale much longer than 1 ps, outside the range of interest. This is because the transfer rate depends on the bond-bond distance (r) as $1/r^6$ (*16*), and r for dangling OH is larger than the nearest-neighbor distance between bonded OH, considering that there is only a quarter monolayer of them at the interface (*8*).

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Phase Separation of Lipid Membranes Analyzed with High-Resolution Secondary Ion Mass Spectrometry

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Lateral variations in membrane composition are postulated to play a central role in many cellular events, but it has been difficult to probe membrane composition and organization on length scales of tens to hundreds of nanometers. We present a high-resolution imaging secondary ion mass spectrometry technique to reveal the lipid distribution within a phase-separated membrane with a lateral resolution of ~100 nanometers. Quantitative information about the chemical composition within small lipid domains was obtained with the use of isotopic labels to identify each molecular species. Composition variations were detected within some domains.

Imaging and quantifying the static and dynamic variations in lateral composition that result from interactions among membrane components is a major challenge in structural biology. Although biological membranes are fluid structures and fluidity is essential for function, it is widely believed that some degree of lateral organization is present and that this organization is also essential for function (*1*–*3*). The relevant distance scale is larger than that of individual membrane proteins or protein assemblies (>10 nm), whose structures can be determined by x-ray crystallography or inferred from atomic force microscopy (AFM), but is substantially below the diffraction limit of light microscopy. Fluorescence microscopy is widely used and is extremely sensitive and specific to the labeled component (*4*–*8*), but only the labeled component is observed, and, at least for relatively small components such as lipids, the fluorophore may greatly alter the delicate interactions that are present in the membrane (*9*). Infrared (*10*) and coherent anti–Stokes Raman (*11*) imaging offer greater chemical specificity, but thus far the lateral resolution and sensitivity are limited. AFM provides much better resolution of topographical features but does not yield information on chemical composition (*9*, *12*–*14*). Imaging mass spectrometry offers distinct advantages over these methods (*15*–*21*), and we applied this approach to imaging and analyzing the chemical composition of small lipid domains with lateral resolution of ~100 nm.

Secondary ion mass spectrometry (SIMS) was performed with a NanoSIMS 50 (Cameca Instruments, Courbevoie, France). During NanoSIMS analysis, a focused $^{133}Cs^+$ primary ion beam is rastered across the sample; secondary ions generated by sputtering are extracted and analyzed according to their respective charge-to-mass ratios at high mass resolving power (Fig. 1). By selectively incorporating a distinctive stable isotope into each membrane component [e.g., ^{13}C or ^{15}N], NanoSIMS secondary ion images characteristic of each species [e.g., $^{13}C^1H^-$ or $^{12}C^{15}N^-$, respectively] can be used to create a component-specific compositional map of the sample. We used this approach to demonstrate the ability to image and analyze quantitatively the composition of very small lipid domains within a phase-separated lipid membrane.

Supported lipid bilayers were prepared from vesicles containing equal mole fractions of ^{15}N-labeled 1,2-dilauroylphosphatidylcholine [^{15}N-DLPC, melting temperature (T_m) = –1°C] and ^{13}C-labeled 1,2-distearoylphosphatidylcholine ($^{13}C_{18}$-DSPC, T_m = 55°C), with 0.5 mol % of a fluorescent lipid added to allow the bilayer quality to be evaluated by fluorescence microscopy during sample preparation (*22*). The sample was maintained at 70°C (above the T_m of both lipid components) to ensure complete mixing, both during vesicle and supported bilayer formation on prewarmed (70°C) silicon wafers. The silicon wafers were prepared with a thin (17 nm) SiO_2 layer that facilitated the formation of stable bilayers while permitting charge dissipation during the SIMS analysis,

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and the wafers were patterned with chrome grids to corral the lipid bilayers and provide landmarks on the surface for characterization of the same regions by fluorescence, AFM, and NanoSIMS imaging (*22*, *23*). The homogeneous, supported bilayer samples were slowly cooled to room temperature to induce phase separation (Fig. 1), rapidly frozen, and freeze-dried to remove water without disrupting the lateral organization within the membrane (fig. S1). Note that the lipid bilayers are fully hydrated before being frozen. Before NanoSIMS analysis, the geometries of the gel-phase domains, which are thicker than the fluid-phase regions, were characterized by AFM for subsequent comparison to the NanoSIMS data. As shown in Fig. 2D, the AFM image of the freeze-dried supported lipid bilayer contained domains that extended ~2.0 nm above the neighboring bilayer, in good agreement with the reported height difference (1.8 nm) between gel-phase DSPC and fluid-phase DLPC in a hydrated supported lipid bilayer on mica (*24*, *25*).

For chemical imaging, the $^{13}C^{1}H^{-}$ and $^{12}C^{15}N^{-}$ NanoSIMS secondary ion signals were used to evaluate the distributions of $^{13}C_{18}$-DSPC and ^{15}N-DLPC, respectively, within the supported lipid bilayer. Although other secondary ions with nominal masses of 14 amu ($^{12}C^{1}H_{2}^{-}$) and 27 amu ($^{13}C^{14}N^{-}$) were generated during analysis, the mass resolving power was sufficient to resolve the $^{13}C^{1}H^{-}$ and $^{12}C^{15}N^{-}$ ions from these interfering isobars while maintaining high lateral resolution, which permitted unambiguous identification of the species of interest. The component-specific NanoSIMS secondary ion images in Fig. 2, A to C, show that the bilayer was not homogeneous. Distinct microdomains enriched in $^{13}C_{18}$-DSPC, as evidenced by an increased $^{13}C^{1}H^{-}$ signal and decreased $^{12}C^{15}N^{-}$ signal, were dispersed within a ^{15}N-DLPC–rich matrix. The area occupied by $^{13}C_{18}$-DSPC within the bilayer was lower than that based on the molar ratio of the lipids in the vesicle solution as prepared. This difference in the lipid composition between the vesicle solution and the phase-separated supported lipid bilayer is likely due to selective adsorption of these very different lipid species (*26*).

Close examination of the sizes and shapes of the $^{13}C_{18}$-DSPC–enriched domains observed in the NanoSIMS secondary ion images revealed that they were nearly identical to the domain geometry imaged by AFM at the same sample locations (Fig. 2D). Phase-separated domains with complex edge structures and domains as small as ~100 nm in diameter, as measured by AFM, are visible in the NanoSIMS secondary ion images (Fig. 2, circles), confirming the high lateral resolution and sensitivity of the NanoSIMS technique. A few of the features in the AFM image did not produce lipid-specific secondary ion signals (Fig. 2, arrows); the height difference between these features and the bilayer (>5 nm, measured by AFM) confirmed that these objects were unlabeled debris and not lipid domains.

Quantitative information on the lipid composition within specified regions of the bilayer was obtained by calibrating the secondary ion yields against standard samples (*22*). Briefly, NanoSIMS measurements were made on sets of homogeneous supported lipid bilayers that systematically varied in the $^{13}C_{18}$-DSPC or ^{15}N-DLPC content, and calibration curves were constructed that correlated the normal-

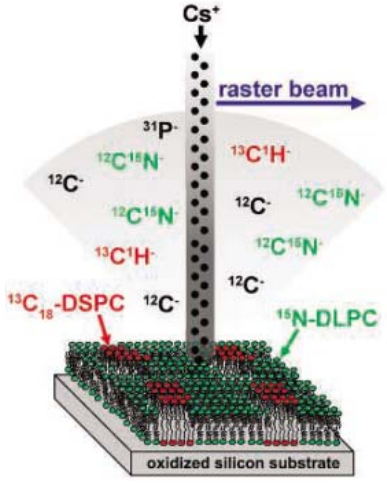

Fig. 1. Schematic showing NanoSIMS analysis of phase-separated lipid bilayer (not to scale). At room temperature, gel and fluid phases, mostly composed of $^{13}C_{18}$-DSPC (red) and ^{15}N-DLPC (green), respectively, are present in the bilayer. The gel phase is ~2 nm higher than the neighboring fluid phase and can be imaged by AFM (compare with Fig. 2D). The sample is freeze-dried to preserve the lateral organization within the bilayer (fig. S1) and analyzed with the NanoSIMS. During NanoSIMS analysis, a focused $^{133}Cs^{+}$ ion beam generates secondary ions; the negative ions are collected and analyzed in a high-resolution mass spectrometer. The secondary ions that are characteristic of $^{13}C_{18}$-DSPC and ^{15}N-DLPC ($^{13}C^{1}H^{-}$ and $^{12}C^{15}N^{-}$, respectively) are used to identify each component in the NanoSIMS image. The $^{133}Cs^{+}$ primary ion beam is focused to a spot ~100 nm in diameter (fig. S2) and is rastered across the sample to generate an image.

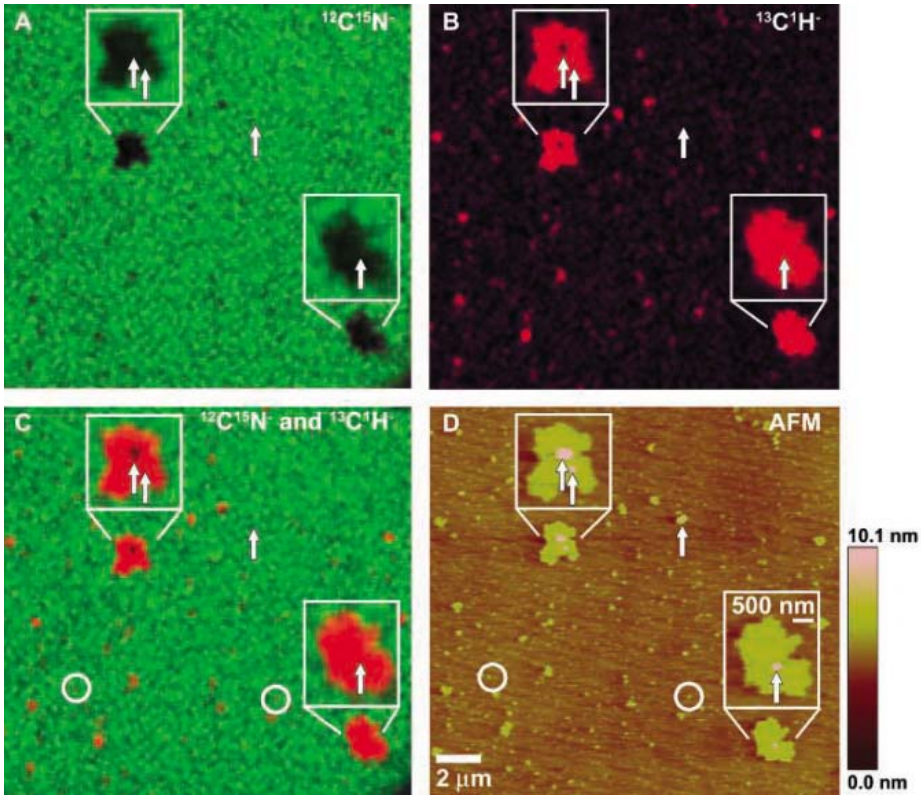

Fig. 2. A phase-separated supported lipid bilayer that was freeze-dried and imaged by NanoSIMS and AFM. (**A** to **C**) NanoSIMS images of the normalized $^{12}C^{15}N^{-}$ signal that localizes ^{15}N-DLPC (A), the $^{13}C^{1}H^{-}$ signal that localizes $^{13}C_{18}$-DSPC (B), and the overlaid $^{12}C^{15}N^{-}$ and $^{13}C^{1}H^{-}$ signals (C). (**D**) An AFM image of the same region on the sample taken before NanoSIMS analysis. The contrast levels within the NanoSIMS images reflect the normalized signal intensity, corresponding to 100 and 0 mol % of the appropriate isotopically labeled lipid, as determined from calibration curves [see text and (*22*)]. Arrows indicate objects in the AFM images that are unlabeled debris, not domains, and their corresponding locations in the NanoSIMS images. Domains with diameters as small as ~100 nm, as measured by AFM, were visible in the SIMS images (e.g., those highlighted with circles). NanoSIMS images were acquired with a pixel size of ~100 nm by 100 nm.

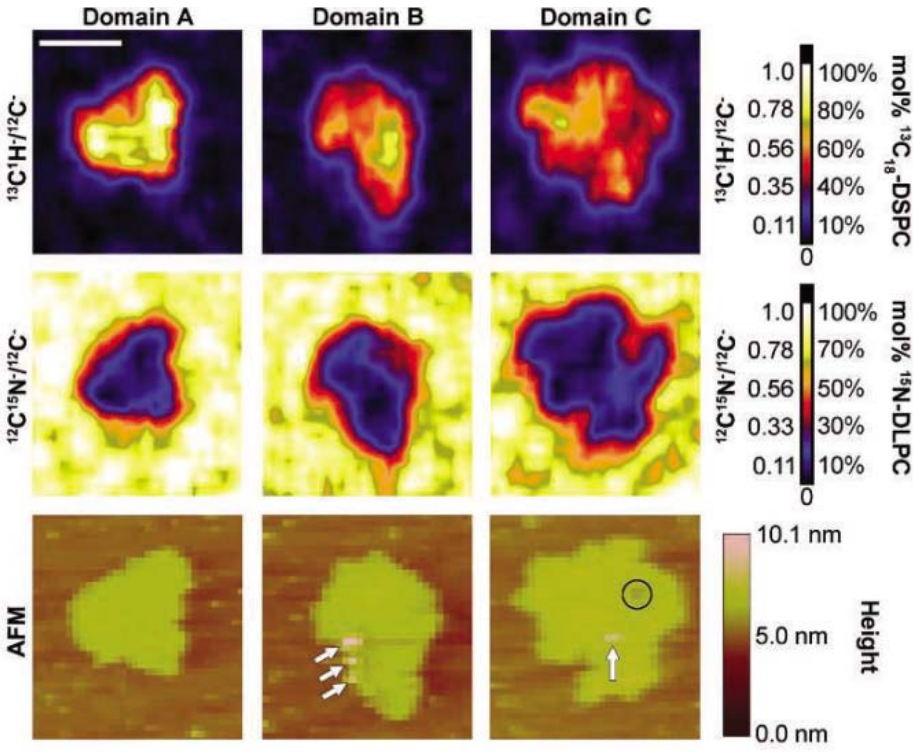

Fig. 3. Details of correlated NanoSIMS and AFM images showing domain composition and topography. The $^{13}C^1H^-/^{12}C^-$ and $^{12}C^{15}N^-/^{12}C^-$ NanoSIMS isotope ratio images show the abundance of $^{13}C_{18}$-DSPC and ^{15}N-DLPC, respectively, within the bilayer, as determined from calibration curves (fig. S3). AFM images acquired at the same sample locations reveal topography. Lower concentrations of both lipids were detected in the locations where debris was identified (arrows). The lipid composition within the gel phase was usually consistent with the phase diagram predictions (domain A), but elevations in the amount of ^{15}N-DLPC within the gel phase were occasionally detected at localized areas within the domains (domains B and C). AFM imaging indicated a small (< 200 nm) depression that could be a fluid-phase subdomain (circle) trapped within the gel phase (domain C); this is confirmed by the NanoSIMS image, which shows an elevated amount of ^{15}N-DLPC across this region (see also Fig. 4). NanoSIMS images were acquired with a pixel size of 100 nm by 100 nm and are smoothed over three pixels. Scale bar, 1 μm.

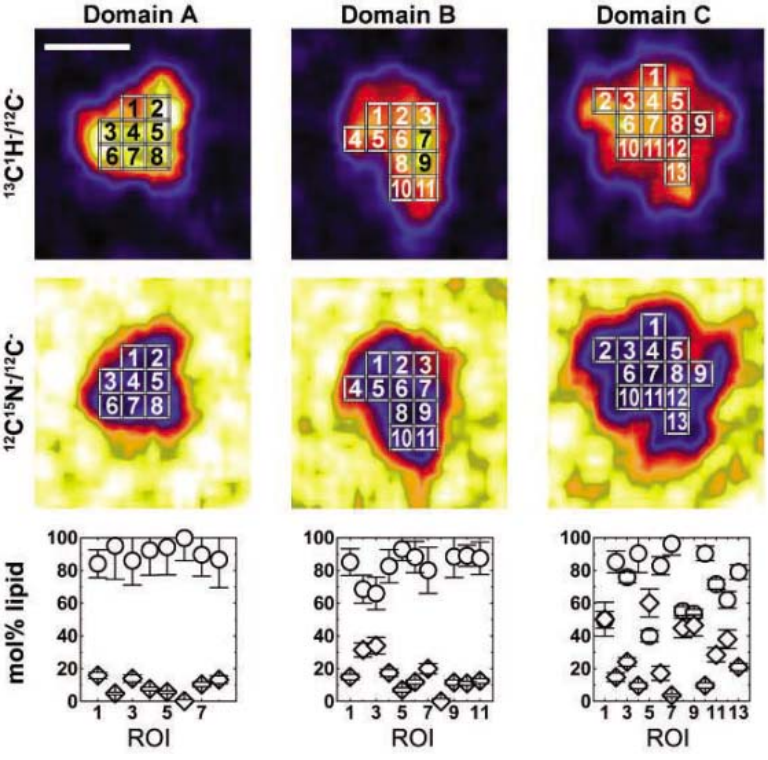

Fig. 4. Quantitative analysis of the gel-phase domains shown in Fig. 3. Each gel-phase domain was divided into regions of 3 pixels by 3 pixels; specific regions of interest (ROIs) within each domain are shown on the NanoSIMS images. The graphs illustrate the amount of $^{13}C_{18}$-DSPC (○) and ^{15}N-DLPC (◇) detected within each domain for the numbered ROI, where each data point represents a region of 3 pixels by 3 pixels within a domain, and the error bars represent the uncertainty calculated from counting statistics (fig. S4) (*22*). Statistically significant lateral variations in lipid composition were detected in domain C, where ROIs 5, 8, and 9 are in the vicinity of the fluid-phase subdomain that was detected by AFM (Fig. 3, domain C, circled region). NanoSIMS images were acquired with a pixel size of 100 nm by 100 nm and are smoothed over three pixels. Scale bar, 1 μm.

ized $^{13}C^1H^-$ or $^{12}C^{15}N^-$ signal intensities ($^{13}C^1H^-/^{12}C^-$ or $^{12}C^{15}N^-/^{12}C^-$) to the mol % of $^{13}C_{18}$-DSPC or ^{15}N-DLPC, respectively, within each sample (fig. S3). With this approach, the gel-phase lipid composition and uniformity were investigated by converting the component-specific secondary ion intensities collected at numerous locations within a single micrometer-sized domain into mol % concentrations (Fig. 3).

We could often detect compositional heterogeneity within the gel phase. Although the majority of the domain consisted of a ~9:1 mol ratio of $^{13}C_{18}$-DSPC to ^{15}N-DLPC, as predicted by the phase diagrams for DSPC and DLPC mixtures (*27*–*29*), higher concentrations of ^{15}N-DLPC were occasionally detected within the gel phase. To determine whether the ^{15}N-DLPC distribution within the gel-phase domains varied in a statistically significant manner, we divided each domain into regions of 3 pixels by 3 pixels that did not include the domain edges or debris (Fig. 4) and used the calibration curves to determine the amount of ^{15}N-DLPC within each region. The variations in the ^{15}N-DLPC content within domains B and C (Fig. 4) were greater than the uncertainty in the measurements, which indicates that these domains contained statistically significant differences in lipid composition. AFM imaging revealed that the elevated ^{15}N-DLPC concentration localized within one $^{13}C_{18}$-DSPC–enriched domain (Figs. 3 and 4, domain C) corresponded to a small (diameter <200 nm) fluid-phase subdomain within the gel phase (Fig. 3, circle). We hypothesize that

small gel-phase domains (tens of nanometers in diameter) that form early in the phase separation process coalesced around a small amount of ^{15}N-DLPC, thereby trapping the fluid-phase subdomain within the growing gel-phase domain [see (*30*) for a theoretical model that may be relevant to this process]. A similar process may have produced the elevated concentrations of ^{15}N-DLPC that were detected at localized regions within other gel-phase domains; however, the absence of topographical features that are characteristic of gel-fluid interfaces at these regions implies that either the fluid-phase subdomains were smaller than the lateral resolution of these AFM images, or the ^{15}N-DLPC was well dispersed within these small regions of the gel phase. [Note that the lateral resolution of these AFM images, ~70 nm, is significantly lower than the highest resolution attainable with AFM because relatively large areas (35 μm by 35 μm) were imaged at 512 pixels by 512 pixels to locate regions for NanoSIMS analysis.] Lower concentrations of both lipids were measured in regions where debris is visible in the AFM images, which indicated that the nonlipid particles were embedded in the bilayer and could have served as nucleation sites. In the fluid phase, the lipid composition was again well approximated by phase diagrams (*27*, *29*); the ratio of ^{15}N-DLPC to $^{13}C_{18}$-DSPC was greater than 19:1, although tiny gel-phase domains scattered throughout the fluid phase may have been included in this value.

With the use of component-specific secondary ion imaging performed with the NanoSIMS, domains as small as ~100 nm in diameter were successfully imaged within a phase-separated lipid membrane, the lipid composition within small regions of the bilayer were quantified, and heterogeneous lipid distributions within gel-phase domains were identified. This example of phase-separated membrane domains also demonstrates the advantage of combining quantitative lipid composition analysis performed by the NanoSIMS with multiple imaging modalities. Because supported lipid bilayers are amenable to isotopic substitution and freeze-drying, this approach can establish the distributions of multiple lipids and membrane-anchored proteins within more complex phase-separated supported membranes by incorporating a distinct stable isotope into each membrane component of interest and simultaneously imaging the secondary ions that distinguish each species. This approach can be extended to living cells by selectively incorporating stable isotopes into membrane components through the use of techniques to label lipid components in live cells (*31*, *32*) as well as by isolating cell membranes with methods to detach intact membrane sheets from live cells (*33*–*36*). In this way, quantitative information on multiple components within native cell membranes may be obtained with high lateral resolution.

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Supporting Online Material

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Ultrafast Carbon-Carbon Single-Bond Rotational Isomerization in Room-Temperature Solution

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Generally, rotational isomerization about the carbon-carbon single bond in simple ethane derivatives in room-temperature solution under thermal equilibrium conditions has been too fast to measure. We achieved this goal using two-dimensional infrared vibrational echo spectroscopy to observe isomerization between the gauche and trans conformations of an ethane derivative, 1-fluoro-2-isocyanato-ethane (1), in a CCl_4 solution at room temperature. The isomerization time constant is 43 picoseconds (ps, 10^{-12} s). Based on this value and on density functional theory calculations of the barrier heights of 1, *n*-butane, and ethane, the time constants for *n*-butane and ethane internal rotation under the same conditions are ~40 and ~12 ps, respectively.

Many molecules can undergo rotational isomerization around one or more of their chemical bonds. During the course of isomerization, a molecule exchanges between relatively stable conformations by passing through unstable configurations. Rotational isomerization is a major factor in the dynamics, reactivity, and biological activity of a multiplicity of molecular structures. Ethane and its derivatives are textbook examples of molecules that undergo this type of isomerization. (*1*) In ethane, as one of the two methyl groups rotates 360° around the central carbon-carbon single bond, it will alternate three times between an unstable eclipsed conformation and the

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preferred staggered conformation. The transition from one staggered state to another leaves ethane structurally identical. Therefore, the result of ethane isomerization cannot be observed through a change in chemical structure. In a 1,2-disubstituted ethane derivative, the molecule can undergo a similar isomerization. However, a 1,2-disubstituted ethane has two distinct staggered conformations, gauche and trans (anti), and two eclipsed conformations, anticlinal and synperiplanar, because of the distinguishing characteristics of the relative positions of the two substituents (Fig. 1A) (*1*). The isomerization is frequently referred to as "hindered internal rotation" (*2*). It has been the subject of intense theoretical and experimental study since Bischoff found 100 years ago that rotation about the C-C single bond in ethane is not completely free. (*2*)

The trans-gauche isomerization of 1,2-disubstituted ethane derivatives, such as *n*-butane, is one of the simplest cases of a first-order chemical reaction. This type of isomerization has served as a basic model for modern chemical reaction kinetic theory and molecular dynamics (MD) simulation studies in condensed phases of matter (*3*–*8*). In spite of extensive theoretical investigation, no corresponding kinetic experiments have been performed to test the results, partially because of the low rotational energy barrier of the *n*-butane (~3.4 kcal/mol) and of other simple 1,2-disubstituted ethane derivatives (*9*). According to theoretical studies (*3*–*8*), the isomerization time scale (1/*k*, where *k* is the rate constant) is 10 to 100 ps at room temperature in liquids.

The room-temperature time scale is much shorter than the microsecond and longer–time scale measurements that can be made with dynamic nuclear magnetic resonance (DNMR) spectroscopy, a widely used method for studying slow temperature-dependent isomerization kinetics (*10*). The picosecond kinetics at room temperature cannot be deduced from microsecond or millisecond dynamics at low temperature because the rate constant is not a simple function of temperature over a wide temperature range (*11*). Thus, DNMR does not afford accurate estimates of isomerization rates at room temperature for molecules with small barriers, such as ethane, that rotate in tens of picoseconds. Other methods to study fast isomerization dynamics under thermal equilibrium, such as linear infrared (IR) and Raman line shape analysis (*12*, *13*), are hampered by multiple contributing factors apart from isomerization (*14*–*16*).

Two-dimensional (2D) IR vibrational echo chemical exchange spectroscopy has recently proven useful for studying fast dynamical processes under thermal equilibrium conditions (*17*–*19*). We applied this method to study the ultrafast trans-gauche isomerization dynamics of a simple 1,2-disubstituted ethane derivative, 1-fluoro-2-isocyanato-ethane (**1**), at room temperature in liquid solution. The experiments were performed by observing the time dependence of the 2D spectrum of the isocyanate group's antisymmetric stretching mode.

Details of the methodology of 2D IR vibrational echo spectroscopy have been described previously (*20*). Very briefly, in a 2D IR vibrational echo experiment, three ultrashort IR pulses tuned to the frequency of the vibrational modes of interest are crossed in the sample. Because the pulses are very short, they have a broad bandwidth that makes it possible to simultaneously excite a number of vibrational modes. The first laser pulse "labels" the initial structures of the species in the sample by setting their initial frequency, ω_τ. The second pulse ends the first time period τ and starts clocking the reaction time period T_w during which the labeled species undergo isomerization and other population dynamics changes such as vibrational relaxation to the ground state and orientational relaxation of the entire molecule. The third pulse ends the population dynamics period of length T_w and begins a third period of length $\leq\tau$, which ends with the emission of the vibrational echo pulse of frequency ω_m. The vibrational echo signal reads out information about the final structures of all labeled species by their frequency ω_m.

There are two types of time periods in the experiment. The times between pulses 1 and 2 and between pulse 3 and the echo pulse are called coherence periods. During these periods, the vibrations are in coherent superpositions of two vibrational states. Fast vibrational oscillator frequency fluctuations induced by fast structural fluctuation of the system cause dynamic dephasing, which is one contribution to the line shapes in the conventional 1D absorption spectrum. During the period T_w between pulses 2 and 3, called the population period, a vibration is in a distinct eigenstate rather than a superposition state. Slow structural fluctuations of the system, termed spectral diffusion, contribute to the 2D line shapes. Other processes during the population period, particularly chemical exchange, also produce changes in the 2D spectrum. Chemical exchange occurs when two species in equilibrium interconvert without changing the overall number of either species. Isomerization back and forth between gauche and trans conformations of **1** is a type of chemical exchange. In other contexts, it has been demonstrated that chemical exchange causes new peaks to grow in as T_w is increased (*17*–*19*). In our experiments, the growth of off-diagonal peaks in the 2D vibrational echo spectrum of **1** with increasing T_w was used to extract the gauche-trans isomerization rate.

The calculated structures and potential energy of **1** as it undergoes rotational isomerization about the central carbon-carbon single bond (Fig. 1, A and B) were obtained using density functional theory (DFT) (*21*) at the B3LYP level and 6-31+G(d,p) basis set for isolated molecules. The energy values were corrected for the zero point energy. The gauche-trans isomerization has two possible transition states: the anticlinal conformer, where the F atom is eclipsed by the H atom; and the synperiplanar conformer, where the F atom is eclipsed by the N atom. Calculations show that the anticlinal conformer is the transition state because it has a much lower energy (3.3 kcal/mol) than the synperiplanar conformer (>7 cal/mol). From the cal-

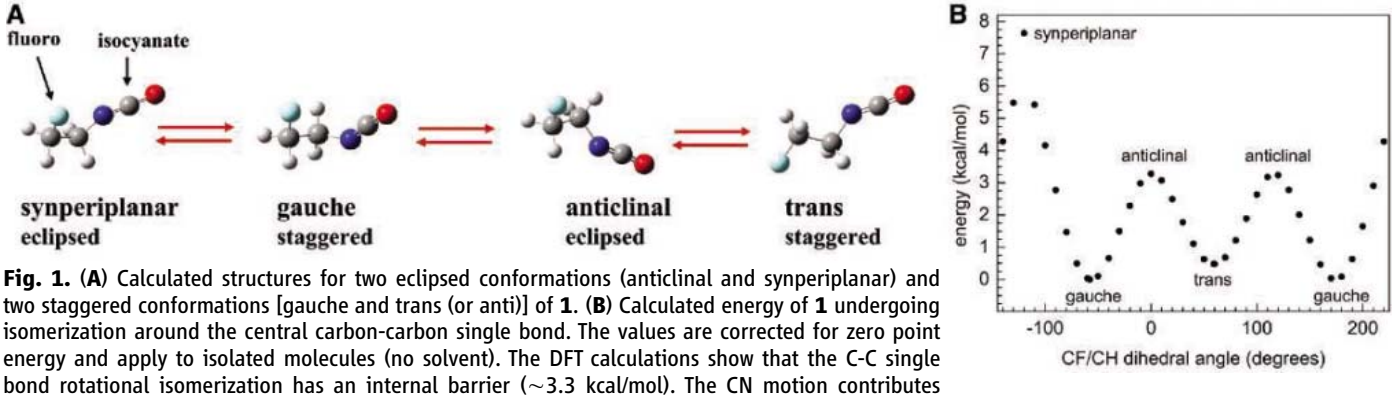

Fig. 1. (**A**) Calculated structures for two eclipsed conformations (anticlinal and synperiplanar) and two staggered conformations [gauche and trans (or anti)] of **1**. (**B**) Calculated energy of **1** undergoing isomerization around the central carbon-carbon single bond. The values are corrected for zero point energy and apply to isolated molecules (no solvent). The DFT calculations show that the C-C single bond rotational isomerization has an internal barrier (~3.3 kcal/mol). The CN motion contributes only slightly to the energy of the configurational change. Thus, the exchange rate between the gauche and trans conformers is essentially the C-C single-bond rotational isomerization rate. The calculations also demonstrate that during exchange between the gauche and trans conformers, the molecule passes through a transition state, the eclipsed conformation (anticlinal), that is similar to the one calculated for ethane isomerization.

culations for the isolated molecule (no solvent), the gauche conformer is about 0.5 kcal/mol more stable than the trans conformer.

The gauche and trans conformers have different geometries and intramolecular interactions, resulting in different vibrational frequencies for the antisymmetric stretching mode of the isocyanate group (NCO) and different dipole moments. Calculations show that the trans conformer has an NCO stretching frequency ~15 cm^{-1} higher than the gauche conformer. In the Fourier transform infrared (FTIR) spectroscopy spectrum of the NCO antisymmetric stretching mode of **1** in CCl_4 at room temperature, there are two peaks of similar intensity (Fig. 2). Based on the calculations, the peak at 2280 cm^{-1} is assigned to the trans conformer and the one at 2265 cm^{-1} to the gauche. The population ratio of trans/gauche (the equilibrium constant) is ~1:1, obtained by analyzing both the 1D and 2D IR data (*19*) (fig. S1). The population ratio was experimentally determined to be temperature- and solvent polarity–dependent, demonstrating that two equilibrated species exist in the system. However, the linear IR spectrum cannot provide information about the isomerization kinetics.

Figure 3A displays six T_w-dependent 2D IR spectra of **1** in a CCl_4 solution at room temperature. The 0-fs panel corresponds to the shortest T_w, at which negligible isomerization has occurred. As discussed in detail previously (*17*), when no isomerization has occurred, the initial and final structures of each labeled species in the sample are unchanged. Therefore, the ω_τ and ω_m values of each peak are identical, and the peaks appear only on the diagonal. The two peaks representing the gauche (ω_m = 2265 cm^{-1}) and trans (ω_m = 2280 cm^{-1}) conformers are clearly visible on the diagonal. After a long reaction period (T_w = 25 ps), isomerization has proceeded to a substantial degree. The obvious change is the additional peak that has appeared at the upper left (ω_τ = 2265 cm^{-1} and ω_m = 2280 cm^{-1}). This peak arises from gauche-to-trans isomerization. There is a corresponding peak to the lower right that is generated by trans-to-gauche isomerization, but it is somewhat negated by a negative-trending peak produced by population relaxation (*22*) between the antisymmetric isocyanate mode and another mode (fig. S1). The negative-trending (blue) peaks due to population relaxation are discussed further below and in the supporting material. They are included in the detailed fitting of the data. The diagonal peaks arise from molecules with the same initial and final structures; that is, molecules that either did not undergo isomerization during the time period T_w or else underwent multiple isomerization cycles that left them in their initial conformation at the end of the T_w period. The growth of the off-diagonal peaks with increasing T_w permits determination of the isomerization rate (chemical exchange rate), although it is necessary to analyze the growth and decay of all the peaks for accuracy (*19*).

During the T_w period, other factors besides chemical exchange also influence the 2D spectrum. These phenomena include spectral diffusion, orientational relaxation, and vibrational relaxation (*17*). Spectral diffusion changes the shape of each peak, and the orientational and vibrational relaxations cause all peaks to decrease in amplitude. Only chemical exchange produces growing off-diagonal positive-trending (red) peaks for the two distinct species. There is an additional vibrational relaxation pathway, distinct from the regular vibrational lifetime decay, that stems from the coupling of the NCO antisymmetric stretch of both conformers to a vibrational mode of unassigned nature at ~2230 cm^{-1} (fig. S1). The coupling induces fast back-and-forth population equilibration between the unassigned mode and the NCO antisymmetric stretch [equilibration time constants are 0.9 ps for the trans conformation and 1.9 ps for the gauche conformation (fig. S2)]. The equilibration via vibrational relaxation also produces additional negative (blue) peaks (*22*) just below each positive (red) peak. As shown at the very bottom of each panel in Fig. 3A, two blue peaks at low frequency along ω_m grow in with T_w. The off-diagonal red peak at ω_τ = 2280 cm^{-1} and ω_m = 2265 cm^{-1} (lower right) is smaller than the upper left off-diagonal peak because of canceling overlap with a negative vibrational relaxation blue peak at approximately ω_τ = 2280 cm^{-1} and ω_m = 2270 cm^{-1}.

Assignment of the growing positive (red) off-diagonal peaks to isomerization, with the one at the lower right offset by an overlapping negative-trending (blue) peak, can be confirmed by examining 1-bromo-2-isocyanato-ethane. The bromo group is so large that it generates substantial steric hindrance in the eclipsed form

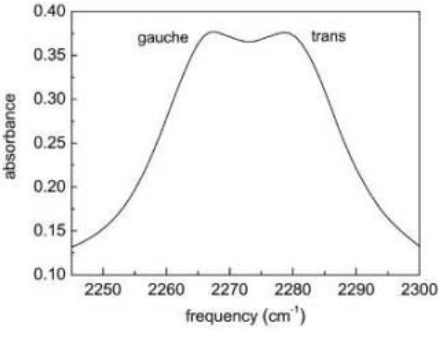

Fig. 2. FTIR spectrum of **1** in a CCl_4 solution at room temperature. Calculations assign the peak at ~2280 cm^{-1} to the trans conformer and the peak at ~2265 cm^{-1} to the gauche conformer.

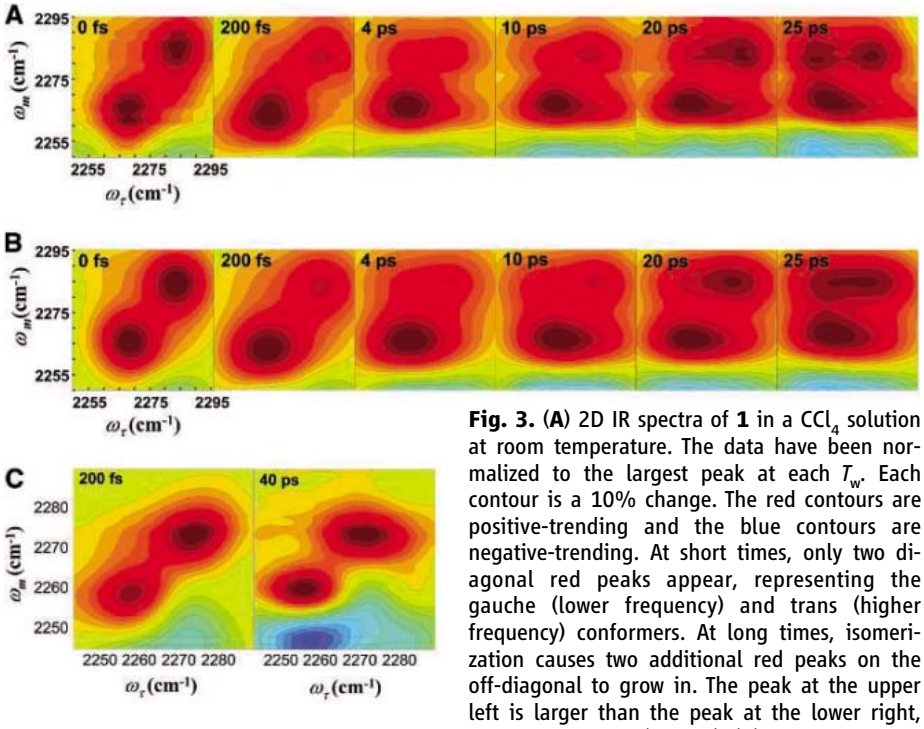

Fig. 3. (**A**) 2D IR spectra of **1** in a CCl_4 solution at room temperature. The data have been normalized to the largest peak at each T_w. Each contour is a 10% change. The red contours are positive-trending and the blue contours are negative-trending. At short times, only two diagonal red peaks appear, representing the gauche (lower frequency) and trans (higher frequency) conformers. At long times, isomerization causes two additional red peaks on the off-diagonal to grow in. The peak at the upper left is larger than the peak at the lower right, because the lower right peak overlaps with a negative-trending peak (Fig. 3C). (**B**) Calculated 2D IR spectra using the model and procedures discussed in the text and in the supporting online material. The agreement between the experiments and the calculated spectra is very good. (**C**) 2D IR spectra of 1-bromo-2-isocyanato-ethane in a CCl_4 solution at room temperature. The bulky bromo group prevents isomerization from occurring past T_w = 40 ps. All other aspects of the system are the same as for **1**. The results show that the positive-trending (red) off-diagonal peaks do not grow in without isomerization. The negative trending peak to the lower right that interferes with the positive-trending isomerization peak in Fig. 3A is apparent.

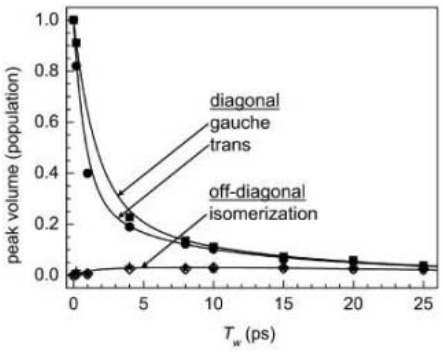

Fig. 4. Data (points) and calculated curves for the 2D spectra, some of which are shown in Fig. 3A. The points are the T_w-dependent peak volumes for the two diagonal and two off-diagonal peaks. The solid curves are all calculated with a single adjustable parameter, the isomerization time constant. The agreement is very good and yields an isomerization time constant of 43 ps. Input parameters of the model, all measured experimentally, are the equilibrium constant $K_{eq} = 1$; orientational relaxation time constants $\tau_T = 3.2$ ps (T, trans) and $\tau_G = 3.4$ ps (G, gauche); and vibrational relaxation time constants $T_T^{fast} = 0.91$ ps with normalized amplitude 0.63, $T_T^{slow} = 18.8$ ps with normalized amplitude 0.37, $T_G^{fast} = 1.95$ ps with normalized amplitude 0.6, and $T_G^{slow} = 18.4$ ps with normalized amplitude 0.4.

and greatly raises the barrier for isomerization. On the time scale of interest here, isomerization does not occur. All other aspects of the system are the same, including the vibrational population equilibration with the unassigned peak. Figure 3C displays 2D IR spectra for a 1-bromo-2-isocyanato-ethane/CCl_4 solution at room temperature at short and long T_w. At 40 ps, no positive-trending (red) off-diagonal peaks have appeared, indicating that the isomerization time constant is much greater than 100 ps. In the absence of the positive-trending off-diagonal isomerization peaks, the negative-trending population equilibration peak that interferes with the lower right isomerization peak in the spectrum of **1** is clearly visible.

To quantitatively model the 2D spectra and extract the kinetic parameters from the 2D IR data, a modified version of the kinetic model described previously was used (*17*, *19*). The trans and gauche conformers undergo isomerization, which results in chemical exchange in the spectra. The amplitudes of the signals for each species decay because of orientational relaxation, fast vibrational relaxation (vibrational equilibration), and slower vibrational lifetime decay to the ground state (fig. S3). The kinetic model requires as inputs the orientational relaxation times and the fast (equilibration) and slow (decay to the ground state) vibrational relaxation times to model the data and obtain the isomerization rate constant. The orientational relaxation and vibrational relaxation time constants were measured with polarization-selective pump-probe experiments (*19*, *23*) (fig. S2). The equilibrium constant ($K_{eq} = 1$) and the vibrational transition dipole moment ratio (~1) were obtained by analyzing both the 1D IR spectrum and the 2D IR spectrum at $T_w = 0$ fs (fig. S1). (*19*) The time-dependent populations are provided by the peak volumes of the four red peaks in 2D IR spectra scaled with the transition dipole moment ratio. Therefore, only one unknown parameter, the isomerization rate constant $k_{TG} = k_{GT}$, was used in the fitting. The trans-to-gauche (TG) and gauche-to-trans (GT) rate constants are taken to be equal within experimental error because the equilibrium constant is 1.

Figure 3B shows calculated 2D spectra using the known input parameters and the results of fitting k_{TG}. Both the measured and calculated spectra are normalized by scaling to make the largest peak at each time equal to unity. Given the complexity of the system, the model calculations do a very good job of reproducing the time-dependent data. Of particular importance is the growth of the off-diagonal red peaks and the negative-trending peaks at the bottom of each panel. The data are well fit using the isomerization rate constant as the only adjustable parameter (Fig. 4). The off-diagonal peaks grow in at the same rate, consistent with $k_{TG} = k_{GT}$ within experimental error. The fits yield $1/k_{TG} = 1/k_{GT} = 43 \pm 10$ ps. The error bars arise from the uncertainty in the parameters that go into the calculations.

Based on the experimental results for the 1-fluoro-2-isocyanato-ethane, it is possible to calculate approximately the gauche-trans isomerization rate of *n*-butane and the rotational isomerization rate of ethane under the same conditions used in this study (CCl_4 solution at room temperature, 297 K). We have analyzed *n*-butane because there is a large number of theoretical calculations for the isomerization of this molecule (*5*–*8*). Transition state theory (*11*) was employed, with the assumption that the prefactors for all of the systems are the same. This assumption is reasonable because the transition states and the barrier heights are quite similar for the three systems. We performed DFT calculations on all the systems using the same method [the B3LYP level and 6-31+ G(d,p) basis set] to obtain the barrier heights. With the zero point energy correction, the trans-to-gauche isomerization of *n*-butane has a barrier of 3.3 kcal/mol. The barrier for ethane is calculated to be 2.5 kcal/mol. This value differs from the 2.9 kcal/mol (*24*, *25*) that has been obtained using more extensive electronic structure calculations. However, here we will employ the 2.5 kcal/mol value for comparison with the 3.3 kcal/mol obtained for **1**. By calculating the two barriers with the same method, there should be some cancellation of errors.

Using the calculated barriers for **1** and for *n*-butane and the assumption that the prefactors are the same, we obtain a ~40 ps time constant for the *n*-butane trans-to-gauche isomerization time constant ($1/k_{TG}$). Twenty-six years ago, Rosenberg, Berne, and Chandler reported a 43-ps time constant for this process (in CCl_4 at 300 K) from MD simulations. (*6*) Other MD simulations gave isomerization rates in liquid *n*-butane at slightly lower temperatures: 52 ps (292 K) (*7*), 57 ps (292 K) (*5*), 50 ps (273 K) (*5*), and 61 ps (<292 K) (*8*). All of these values are reasonably close to the value obtained here based on the experimental measurements of **1**. In the same manner, the isomerization time constant for ethane is found to be ~12 ps. This value can be improved by better electronic structure calculations on **1** and calculations for both **1** and ethane that include the CCl_4 in determining the barriers.

This 2D IR vibrational echo technique should be generally applicable to the study of fast isomerizations, most of which give rise to conformers with necessarily distinct vibrational spectra. For the method to be useful, the isomerization time constant must fall into a time window determined by experimental considerations. The lower time limit of the method is determined by the laser pulse duration, which can be <50 fs and therefore is not a serious restriction. The upper time limit is determined by the vibrational lifetime of the mode used to probe the isomerization. In practice, the isomerization time constant should be less than three times the vibrational lifetime. Some modes, such as the CO stretches of metal carbonyl compounds, can have lifetimes of hundreds of picoseconds. Within the experimental limitations, the method used here should be useful for addressing a variety of important problems.

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Soluble Mn(III) in Suboxic Zones

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Soluble manganese(III) [Mn(III)] has been thought to disproportionate to soluble Mn(II) and particulate $Mn^{IV}O_2$ in natural waters, although it persists as complexes in laboratory solutions. We report that, in the Black Sea, soluble Mn(III) concentrations were as high as 5 micromolar and constituted up to 100% of the total dissolved Mn pool. Depth profiles indicated that soluble Mn(III) was produced at the top of the suboxic zone by Mn(II) oxidation and at the bottom of the suboxic zone by $Mn^{IV}O_2$ reduction, then stabilized in each case by unknown natural ligands. We also found micromolar concentrations of dissolved Mn(III) in the Chesapeake Bay. Dissolved Mn(III) can maintain the existence of suboxic zones because it can act as either an electron acceptor or donor. Our data indicate that Mn(III) should be ubiquitous at all water column and sediment oxic/anoxic interfaces in the environment.

Manganese is essential in a variety of biogeochemical processes from photosynthesis to bacterially mediated organic matter decomposition (*1*, *2*). It acts as a catalyst in an important microbially mediated redox cycle to oxidize and detoxify H_2S; Mn(II) is first oxidized by O_2 to MnO_2 (*3*, *4*), which then oxidizes H_2S with re-formation of Mn(II) (*5*–*8*). Traditionally, dissolved manganese (material passing through 0.2- or 0.4-μm filters) has been assumed to be Mn(II), whereas particulate manganese has been assumed to exist only as MnO_2 because any inorganic Mn(III) formed would disproportionate to Mn(II) and Mn(IV). However, soluble Mn(III) can be stabilized with organic chelates and inorganic chelates (e.g., pyrophosphate, a particularly good chelating agent) to prevent disproportionation and is well known in laboratory solutions (*9*–*14*). Because its existence as a major chemical species has not been documented in the aquatic environment, soluble Mn(III) has become an overlooked Mn species.

Because the d_{z^2} and $d_{x^2-y^2}$ orbitals that can accept electrons in MnO_2 or donate electrons from Mn(II) are spatially distinct, reduction of MnO_2 or oxidation of Mn(II) should lead to Mn(III) species via one-electron transfer processes (*15*). Possible natural sources of soluble Mn(III) include photosynthetic reaction centers released during phytoplankton decomposition, as well as its formation as an intermediate in the bacterial oxidation of Mn(II) → Mn(IV) (*16*) and reduction of Mn oxides with sulfide (*17*). The Mn(III) formed can then be complexed by a variety of ligands that include pyrophosphate (formed from the breakdown of adenosine 5′-triphosphate or adenosine 5′-diphosphate), siderophores that bind extracellular Fe(III) and Mn(III) with similar binding strengths, or other natural ligands (*9*–*14*).

Once formed, Mn(III) is an important one-electron transfer redox species that can act as either an oxidant or a reductant. Mn(III) is an ideal chemical species to maintain the existence of suboxic zones, which have dissolved O_2 and H_2S below normal detection levels. Suboxic zones are ubiquitous as they are found in sedimentary porewaters of lakes, estuaries, bays, and oceans; in permanently anoxic basins (the Black Sea, the Arabian Sea, equatorial Pacific, and fjords); and in shallower seasonally anoxic basins (the Chesapeake Bay and Saanich Inlet).

To test the hypothesis that soluble Mn(III) is present and a key redox species in the Mn catalytic redox cycle, we used known Mn(III) coordination chemistry to search for Mn(III) in the suboxic waters of the Black Sea and the Chesapeake Bay. We used in situ voltammetry (*18*–*20*) to simultaneously measure O_2 and H_2S (defined as the sum of H_2S, HS^-, S_x^{2-}, and S_8) in one cast. Once the suboxic zone was documented, we used traditional bottle methods on a subsequent cast to obtain samples for total dissolved Mn, particulate Mn (*21*), and dissolved Mn(III). Dissolved Mn is defined as that material which passes through 0.2-μm Nuclepore filters. This approach can separate soluble Mn(III) from particulate MnO_x. Previous marine and estuarine studies (*22*, *23*) that used radiotracer Mn showed that all size-fractionated particulate MnO_x is trapped on 1.0-μm or smaller filters as Mg^{2+} and Ca^{2+} induce MnO_2 precipitation (*24*). X-ray absorption near-edge spectroscopy measurements (*25*) have documented that particulate MnO_x from Black Sea waters, formed by microbially mediated Mn(II) oxidation, is in the Mn(IV) state and similar to δ-MnO_2.

To measure Mn(III) in field samples, we used cathodic stripping voltammetry to detect Mn(III) as the known desferrioxamine-B (DEF-B) complex (*9*). Samples were collected in gas-tight syringes directly from a Niskin bottle and then filtered in an argon-filled glove bag. After running background voltammograms to determine that H_2S and O_2 were not measurable in the sample, we added DEF-B as a competitive ligand to 10 ml of sample so that the total concentration was 20 μM. DEF-B gives a ligand peak at –1.34 V versus saturated calomel electrode (SCE), and the Mn(III)DEF-B complex has a signal at –1.19 V versus SCE (Fig. 1). This procedure was calibrated with 1 to 5 μM Mn(III)pyrophosphate standards (fig. S1). DEF-B complexed all dissolved Mn(III) in standards and in samples within 30 s of mixing, indicating that DEF-B complexed Mn(III) more strongly than pyrophosphate, as expected on the basis of the known stability constants of

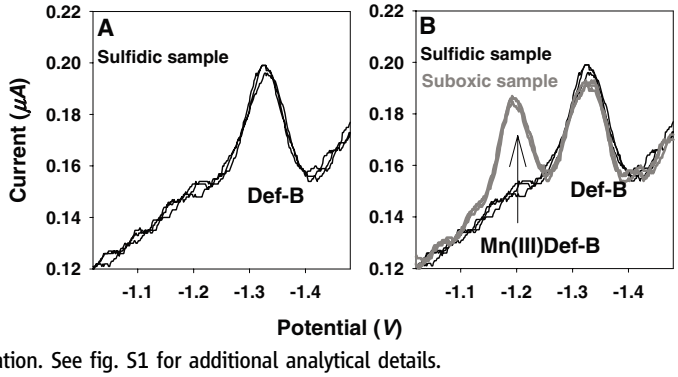

Fig. 1. (**A**) Free desferrioxamine-B (DEF-B) ligand signal at −1.34 V in a sample without Mn(III) complexes (oxic or sulfidic sample). (**B**) Mn(III)DEF-B complex signal at −1.19 V and the free ligand signal at −1.34 V in a sample from the suboxic region of the Black Sea. The free-ligand DEF-B peak decreased due to complexation. See fig. S1 for additional analytical details.

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Mn(III) with pyrophosphate and DEF-B (*10*, *14*) and other natural ligands. The Mn(III)DEF-B and Mn(III)pyrophosphate complexes are stable but are reduced by H_2S to Mn(II), as confirmed by voltammetric and spectroscopic measurements. To confirm that only dissolved Mn(III) was measured, we added a colloidal form of soluble manganese dioxide (*24*) to Black Sea (and Chesapeake Bay) waters and then added DEF-B to the sample. No Mn(III)DEF-B production was observed during the 4-hour measurement period.

Measurements of dissolved Mn(III) were made during a month-long Black Sea research cruise on the *R/V Knorr* in 2003. The cruise had three legs; leg 172-07 (15 to 25 April), leg 172-08 (25 April to May 10), and leg 172-09 (10 to 15 May). Twelve stations in the Black Sea (Fig. 2A) were analyzed for soluble Mn(III) complexes. Several stations were sampled two times on different days, and one station in the central west of the Black Sea was sampled six times over the expedition. The water column of the southwest Black Sea was sampled more intensively because highly saline water enters the Black Sea through the Bosporus Strait and mixes with lower salinity oxygen-rich cold intermediate-layer (CIL) waters. The saline intrusions are pushed eastward by western gyre waters, which have a cyclonic circulation pattern (*20*), and disrupt the thickness of the suboxic zone (defined as $O_2 < 3$ μM and $H_2S < 0.2$ μM).

In each Black Sea depth profile (Fig. 2, B to F; fig. S2), O_2 concentration decreased with depth and density, as a result of organic matter decomposition with O_2 as electron acceptor, to the suboxic zone containing no detectable O_2 and ΣH_2S. ΣH_2S concentrations increased below that zone. The suboxic zone in the eastern and central Black Sea had a thickness of at least 0.50 potential density (σ_t) units (Fig. 2F) (σ_t = 15.61 to 16.13). However, the thickness decreased on proximity to the Bosporus because the west central Black Sea (Fig. 2B, site 9) is ≤ 0.44 σ_t units (15.58 to 16.02) and southwest stations are thinner (e.g., Fig. 2E, site 4, σ_t = 15.80 to 16.15).

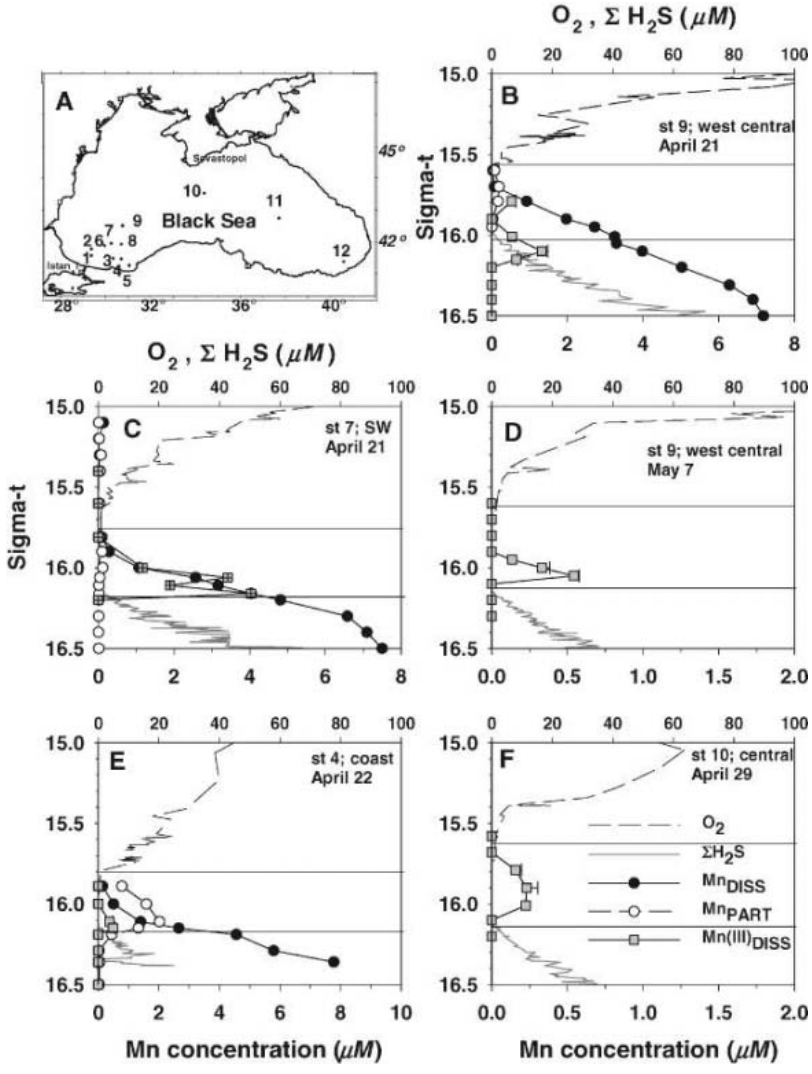

Fig. 2. Map of the Black Sea (**A**), and representative profiles of ΣH_2S, O_2, Mn(III) organic complexes, $Mn_{dissolved}$, and $Mn_{particulate}$ at different stations in the west central (**B** and **D**), southwest (**C** and **E**), and central Black Sea (**F**). ΣH_2S is the sum of H_2S/HS^-, S(0) as S_8, and S_x^{2-}. Particulate and total dissolved manganese measurements for a revisit to site 9 and site 10 (D and F) were not plotted, because those measurements were conducted on a different cast from the dissolved Mn(III) measurements. To compare results at different stations, we plotted chemical species versus potential density (σ_t), and not depth, because the appearance or disappearance of chemical species in the Black Sea follows characteristic density surfaces (*29*). For example, O_2 is last detected at an average σ_t of 15.60, and H_2S is first detected at an average σ_t of 16.15. But O_2 was detected deeper (down to 15.95) in the southwest, where oxygen-rich water intrudes and is pushed eastward by the cyclonic circulation. The suboxic zone thickness ranges from 20 to 40 m. The area between the horizontal lines in (B) to (F) indicates the extent of the suboxic zone for each profile.

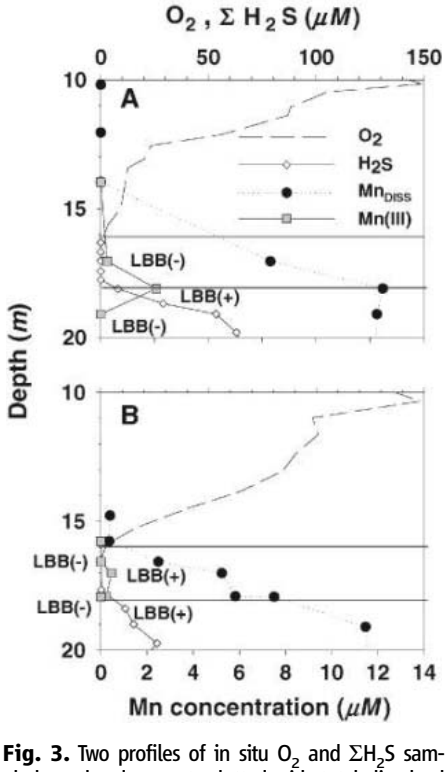

Fig. 3. Two profiles of in situ O_2 and ΣH_2S sampled on the downcast, plotted with total dissolved manganese and dissolved Mn(III) complexes on water sampled from the upcast. Tidal influences can affect the thickness of the suboxic zone within a complete cast. For these casts, the in situ analyzer and the bottles were on the same CTD rosette system. On the upcast, bottle samples and the in situ analyzer did not have a ΣH_2S signal when soluble Mn(III) was detected. (**A**) Cast taken on 3 August 2003 at 19:38 and (**B**) 4 August 2003 at 14:02 and 17:14. LBB(+) and LLB(−) indicate a positive and negative result for leucoberbelin blue (LBB), respectively. The area between the horizontal lines indicates the extent of the suboxic zone for each profile.

Particulate manganese concentrations were typically low (<0.2 μM), except near the coastal zone (Fig. 2E, site 4) where maximum concentrations were 2 μM. Coexistence of oxidized particulate and dissolved manganese and H_2S was not observed in samples from bottles. The apparent overlap of oxidized Mn with the in situ sulfide signal (Fig. 2B) is due to soluble S(0) (*19*) and because the samples came from different casts [additionally, the conductivity-temperature-depth (CTD) sensor and the bottles are offset by 0.5 m; for the cast in Fig. 2B, the σ_t range of 16.00 to 16.15 had a vertical-depth resolution of 6 m versus 13 m for that in Fig. 2D]. Dissolved manganese was present in all samples of the Black Sea's suboxic region, with a maximum concentration of ~8 μM below the H_2S onset (Fig. 2, C and E). Mn(III) complexes were detected at 10 of the 12 investigated stations with the exception of station 2 in the southwest and station 12 in the eastern basin. The highest Mn(III) complex concentrations were measured at stations 6 (5 μM; figs. S2 and S3) and 7 (4 μM; Fig. 2C), where Mn(III) constituted up to 100% of the dissolved Mn at the maximum dissolved Mn concentrations. We conclude that, at these southwestern sites, intrusions of oxygen-rich water from the Bosporus intensified the manganese redox cycle and caused higher production of dissolved Mn(III). In contrast, the central and eastern Black Sea had lower dissolved Mn(III) concentrations, typically just above the detection limit of the analytical method of ~150 nM (Fig. 2F and figs. S2 and S3). For all other suboxic areas, Mn(III) complexes when detected constituted 16 to 56% of the dissolved Mn.

We observed two types of dissolved Mn(III) profiles in the suboxic zone. The more common one (16 of 18 profiles) was a single maximum at the top (Fig. 2, C and D), at the bottom (Fig. 2E), or in the middle (Fig. 2F, fig. S2). The latter was observed at the central and eastern stations 10 and 11, which do not experience lateral O_2 intrusions. The least common profile (2 of 18; e.g., Fig. 2B) showed two distinct maxima at the top and bottom of the suboxic zone and was observed at coastal station 5 (fig. S2C) and west central station 9 (Fig. 2B). The maximum at the top of the suboxic zone (Fig. 2, B to D) occurred just below where O_2 disappeared and as particulate and dissolved manganese started to increase with depth. This maximum is characterized by the one-electron oxidation of Mn(II). Dissolved Mn(III), trapped with chelating agents, is formed during the oxidation of Mn(II) (*16*). Organic matter decomposition with release of photocenter PSII products could also lead to soluble Mn(III). The second maximum occurred at the bottom of the suboxic zone (Fig. 2, B and E), where particulate manganese decreased and the first sulfur species were detected. At this deeper maximum, H_2S reduced MnO_2-forming polysulfides and elemental sulfur [particulate S_8 is 2 μM as S^0; e.g., (*20*)]. The profiles indicate that Mn(III) complexes are formed during the one-electron reduction of MnO_2.

Dissolved Mn(III) profiles (Fig. 2, B and D) measured on the same day (21 April) at the west central site (station 9) were reproducible (not shown), but these profiles were different from the profile measured on 7 May, both in the number of maxima, as well as in maximum Mn(III) complex concentration, 1.4 μM versus 0.6 μM. Winter conditions in March resulted in surface ventilation of the Black Sea's CIL waters (*26*) and persisted past mid-April. The two Mn(III) maxima in April indicate that both Mn(II) oxidation and Mn(IV) reduction occurred and resulted in an active Mn redox cycle. On 7 May, calmer seas reestablished a stable suboxic zone with a less active Mn redox cycle.

We sampled the Chesapeake Bay water column in July 2002, August 2003, and 2004 below the Bay Bridge (8°58.10′ N; 76°21.43′ W). In July 2002, the suboxic region was poorly developed, because a recent storm had mixed the water column. Dissolved Mn(III) complexes were not observed. In contrast, the suboxic zone in 2003 was well developed and had a maximum thickness of 3 m on 3 to 5 August (Fig. 3). We measured six water-column profiles on 3 days (3, 4, and 5 August), and the results confirmed that Mn(III) complexes were present in the suboxic zone of the Chesapeake Bay then. Leucoberbelin blue (LBB) was used to confirm the presence of Mn(III,IV) species and functioned as a secondary indicator for the presence of dissolved Mn(III) complexes in filtered waters (*27*, *28*). The suboxic zone was not stable with depth but moved up and down in the water column with the tidal cycle, as sulfidic bottom waters and oxygenated surface waters were mixed into the suboxic zone.

Typically, filtration has been used to separate the oxidized and the reduced manganese fractions. This method does not discriminate between the presence of soluble Mn(III) complexes and Mn(II), because the formaldoxime reagent reduces oxidized Mn phases that pass through the filter. Earlier studies (*3–5*, *21*, *29*) thus underestimated the oxidizing-reducing capacity of the soluble Mn pool. Also, studies based on these analytical techniques, as well as atomic absorption spectroscopy, underestimated the capacity for microorganisms to affect biogeochemical processes because Mn(III) can be used as either an electron acceptor or donor.

Our data demonstrate that one-electron transfer reactions are more important for Mn biogeochemistry than previously thought. Thus, dissolved Mn(III) will likely be important in other suboxic systems such as plumes from hydrothermal vents and productive rivers, stratified lakes, sedimentary porewaters, and major oxygen minimum zones such as the Arabian Sea and the equatorial Pacific. Depending on the thermodynamic and kinetic stability of the ligands binding Mn(III), Mn(III) should exist at (sub)nanomolar concentrations in oxic waters of lakes and oceans, as has been found for Fe(III) (*30–32*). Mn(III) will also compete with Fe(III) for these ligands (*12*).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5795/1955/DC1
Materials and Methods
Figs. S1 to S3
References

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Satellite Gravity Measurements Confirm Accelerated Melting of Greenland Ice Sheet

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Using time-variable gravity measurements from the Gravity Recovery and Climate Experiment (GRACE) satellite mission, we estimate ice mass changes over Greenland during the period April 2002 to November 2005. After correcting for the effects of spatial filtering and limited resolution of GRACE data, the estimated total ice melting rate over Greenland is −239 ± 23 cubic kilometers per year, mostly from East Greenland. This estimate agrees remarkably well with a recent assessment of −224 ± 41 cubic kilometers per year, based on satellite radar interferometry data. GRACE estimates in southeast Greenland suggest accelerated melting since the summer of 2004, consistent with the latest remote sensing measurements.

Greenland is the location of the second largest ice cap on Earth and contains about 2.5 million cubic kilometers (km^3) or 10% of the total global ice mass (Fig. 1). Complete melting of the Greenland cap would raise global mean sea level by about 6.5 m. Repeat-pass airborne laser altimetry measurements indicate that Greenland lost ice at a significant rate (–80 ± 12 km^3/year) during the period 1997 to 2003 (*1*). Most of the estimated loss comes from the periphery, whereas the interior appears to be in balance. A more recent study (*2*) based on satellite interferometry suggested that ice loss has been accelerating in recent years and was near –224 ± 41 km^3/year in 2005, significantly larger than the estimate (–80 ± 12 km^3/year) from airborne laser altimetry measurements (between 1997 and 2003), and also significantly larger than the estimate (–91 ± 31 km^3/year) from satellite interferometry observations in 1996 (*2*). Acceleration of mass loss over Greenland, if confirmed, would be consistent with proposed increased global warming in recent years and would indicate additional polar ice sheet contributions to global sea level rise (*3*).

We used satellite gravity measurements to estimate mass change over Greenland. Since its launch in March 2002, the NASA–German Aerospace Center Gravity Recovery and Climate Experiment (GRACE) has been providing measurements of Earth's gravity field at roughly monthly intervals (*4*, *5*). After atmospheric and oceanic contributions are removed (through the GRACE dealiasing process) (*6*), monthly gravity field variations mainly reflect changes in terrestrial water storage, snow/ice mass of polar ice sheets, and mountain glaciers. GRACE data have been successfully used to determine seasonal terrestrial water storage change in major river basins (*7*–*9*) and seasonal nonsteric global mean sea level change (*10*, *11*). To use GRACE to study trends in glacial ice mass in polar regions, one must also consider changes that arise from postglacial rebound (PGR), the delayed response of the crust and mantle to past glacial loads (*12*). Because PGR effects are present within the same geographical regions as current deglaciation, a PGR model is required to separate the effects. Based on the ICE5G model (*12*), average PGR effects over all of Greenland are estimated to be small (*13*).

As longer GRACE time series become available, studies of long-term ice mass change in polar ice sheets become possible (*13*–*17*). Previous studies focused mainly on continental scales and have been limited by the spatial resolution of GRACE gravity fields. It is possible to improve the spatial resolution of GRACE estimates somewhat by assuming that surface load variations in the oceans are much smaller than those on land, especially at long periods (*16*, *18*). To improve resolution beyond this, we resorted to numerical simulations to assign mass changes to regions suggested by remote sensing or other observations. We used 40 monthly GRACE gravity fields over a 3.5-year period from April 2002 to November 2005. These are the release-01 GRACE solutions provided by the Center for Space Research, University of Texas at Austin (*6*). Using a two-step optimized filtering technique developed in a recent study (*16*), we fitted linear trends to estimate ice mass rates over the entire Greenland ice sheet. The optimized filtering technique is designed to maximize the signal-to-noise ratio (*18*) in GRACE mass change fields. A separate regional estimate for East Greenland is of particular interest because satellite radar interferometry measurements show significant loss.

A global gridded (1° by 1°) surface mass change field is estimated from each of the 40 GRACE gravity solutions. At each grid point, we estimated from the time series of mass change a linear trend using unweighted least squares, after first subtracting least squares seasonal (annual and semiannual) signals. Figure 2A shows GRACE surface mass rates over Greenland and surrounding regions. Prominent negative trends (about –3 to –4 cm/year of equivalent water height change) are observed over much of Greenland. Spatial leakage effects are also evident, because of filtering applied to suppress the noise in high-degree and high-order spherical harmonics. Two other prominent features are positive rates (mass accumulation) near Hudson Bay and Scandinavia. In these two regions, a strong PGR signal is predicted by models (*12*). Figure 2 shows two regions of mass loss in eastern Greenland. One is in the southeast, where active ice flow and related ice loss are observed by remote sensing and satellite radar altimetry (*1*, *2*), and the other is along the coast in the northeast. As we show below, the region of loss in the northeast can be accounted for by a combination of northeast Greenland loss and additional loss from Svalbard, which shifts the center of the region slightly off the Greenland coast into the oceans.

We selected two grid points (A and B, marked in Fig. 2A), near centers of the mass loss features, and showed the associated time series in Fig. 3. The red lines are linear trends from unweighted least square fits. The GRACE time series for both points A and B show negative trends on the order of –4 to –5 cm/year) superimposed on seasonal variations. At point A, the later portion of the time series shows an increased rate of about –7.24 cm/year, compared with about –1.03 cm/year for the first 2 years (up to July 2004). The rate for the entire 3.5-year period is –4.59 ± 0.39 cm/year. Although these rates need to be adjusted for effects of spatial filtering, it is clear that GRACE

Fig. 1. The Greenland ice sheet is the second largest ice cap on Earth and contains ~2.5 million cubic kilometers, or 10% of total global ice mass.

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has observed accelerated ice mass loss in southeast Greenland in recent years, consistent with recent assessments (*1*) from satellite interferometry measurements.

Figure 2A suggests that limited spatial resolution of GRACE estimates causes a large portion of variance to be spread into the surrounding oceans, even though the actual source location is likely on the continent. Similarly, PGR effects from nearby regions such as Hudson Bay may contribute to variations over Greenland. Numerical simulations can help identify probable mass change sources that are consistent with GRACE observations. These experiments (see SOM Text and fig. S1) consist of proposing probable geographical regions as sources of mass change, applying processing steps replicating the limited spatial resolution of GRACE data, and comparing predictions with GRACE observations.

The predicted gravity data (Fig. 2B) shows a good match with the GRACE observations in Fig. 2A, both over Greenland and in surrounding regions, including the oceans. To assign an uncertainty to this figure, we scaled up errors assigned to linear rates determined from GRACE. The contribution of GRACE measurement error to uncertainty was small, because the rate was estimated from over 3.5 years of observations. Therefore, the estimate for Greenland is –239 ± 23 km^3/year. This figure agrees well with a recent estimate of –224 ± 41 km^3/year from satellite radar interferometry (*2*) and is significantly larger than earlier assessments, about –80 to –90 km^3/year from remote sensing, satellite interferometry, and the first 2 years of GRACE data.

Most of the –239 ± 23 km^3/year simulated loss comes from east Greenland, with about –90 km^3/year from the southeast Greenland glaciers (blue shaded area in fig. S1), consistent with recent satellite interferometry observations (*2*). About –74 km^3/year is assigned to northeast Greenland, where satellite interferometry observations suggest negligible ice mass change. However, Fig. 2A suggests that the loss may come from latitudes above 80°N, within the area marked by the black box on Fig. 1, containing glaciers separate from the main Greenland ice sheet that were excluded from recent interferometry estimates (*2*). Therefore, it is possible that mass loss in this region has been observed by GRACE but is omitted from the interferometry estimates. The "dipole" feature of Greenland mass loss was also suggested by a recent study (*17*).

The numerical simulation also shows that GRACE observations are consistent with significant mass loss (about –75 km^3/year) over Svalbard, where remote sensing estimates are lacking. However, a recent study (*19*), based on gravity and surface deformation observations in Svalbard, suggests significant present-day glacial melting in the region. Absolute gravity measurements indicate a melting rate of about –50 km^3/year, whereas surface deformation data suggest a rate of about –25 km^3/year. The substantial variability among surface deformation, surface gravity, and our GRACE estimate of Svalbard melting can be attributed to many factors, but all suggest that significant glacial melting is taking place, another strong indication of Arctic warming.

To this point, we have neglected PGR effects in the immediate area of Greenland and surrounding regions (circled by the white line in Fig. 2, A and B). This assumption appears to be supported by the estimated total PGR contribution (about –5 km^3/year) over Greenland in a recent study (*13*), based on the ICE5G model (*12*). Different PGR models may show large discrepancies in modeling the Greenland surface deformation effect, which is largely controlled by the ice history and the solid Earth properties (e.g.,

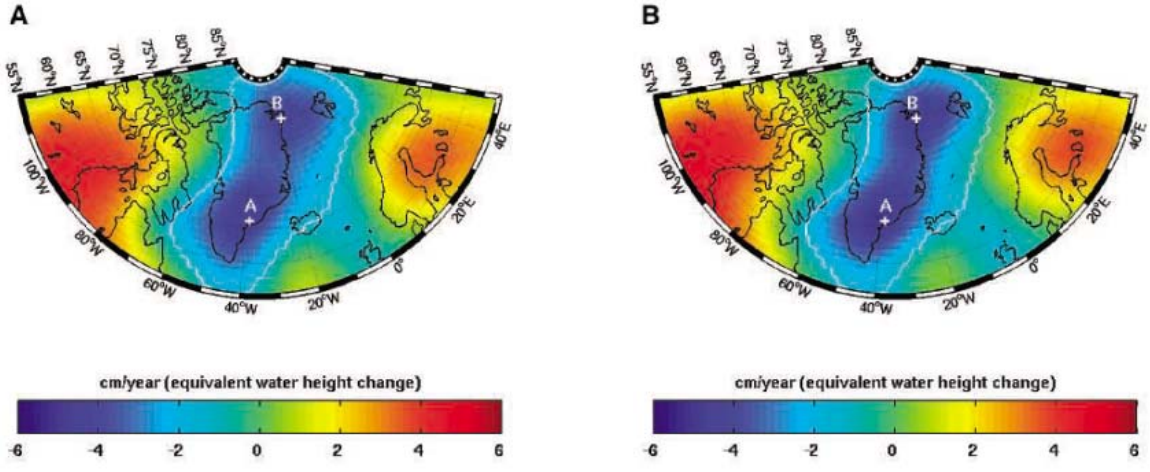

Fig. 2. (**A**) GRACE long-term mass rates over Greenland and surrounding regions during the period April 2002 to November 2005, determined from mass change time series on a 1° grid. (**B**) Simulated long-term mass rates over Greenland and surrounding regions from the experiment as described in SOM text and fig. S1.

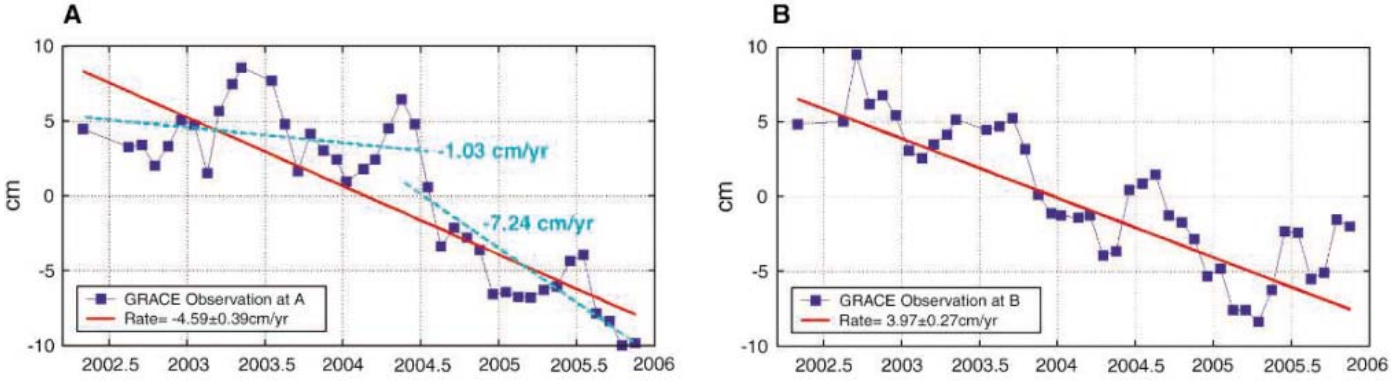

Fig. 3. (**A** and **B**) GRACE mass changes at points A and B in East Greenland, marked on Fig. 2. The straight red lines are long-term linear rates estimated from unweighted least squares fit.

mantle viscosity and crust thickness) in that region, especially over the Hudson Bay and Scandinavia, two prominent PGR active areas. It is possible that the ICE5G PGR model (*13*) may underestimate the PGR contribution to GRACE-observed ice mass loss over Greenland. However, the uncertainty of the estimated PGR contribution will not likely account for a significant portion of the –239 ± 23 km^3/year ice mass loss observed by GRACE. If we adopt this ICE5G-based PGR contribution of mass rate over Greenland (about –5 km^3/year, with uncertainty at 100% of the signal, i.e., ±5 km^3/year), then our GRACE estimate of Greenland ice mass rate is about –234 ± 24 km^3/year.

The current GRACE estimate is significantly larger than an earlier estimate (–82 ± 28 km^3/year), based on just the first 2 years of data (*13*). The difference is attributed both to increased melting in the most recent 1.5-year period and to improved filtering and estimation techniques (including use of numerical simulations), and the latter may have played a more important role. Increased recent melting may represent simple interannual variability or accelerated melting driven by steady Arctic warming (*20*). Despite close agreement between our GRACE estimate and recent radar interferometry estimates (*2*), quantification of Greenland ice mass balance remains a challenge. For example, another study (*21*) based on 10 years of radar altimetry data during the period 1992 to 2002 suggests a small mass gain for Greenland (~11 ± 3 km^3/year) (*2*), opposite in sign to the more recent estimate (*2*). On the other hand, thermomechanical ice models forced by general circulation model climate scenarios predict significant Greenland ice loss in the 21st century (*22*).

The numerical simulation approach used in this study is useful in interpreting GRACE time-variable gravity fields. It contrasts with the basin kernel function approach (*13*, *15*), in which the focus is on a continent-wide average. Numerical simulations are useful in quantifying spatial leakage of variance and in testing hypotheses concerning possible regional contributors to change, such as the Southeast Glacier or Svalbard. Many error sources may affect our GRACE estimates, which include the remaining GRACE measurement error (after spatial smoothing), uncertainty in the background geophysical models used in GRACE (e.g., the uncorrected ocean pole effect in the release-01 GRACE data and errors in the atmospheric and ocean models over Greenland and surrounding regions), and unquantified other leakage effects.

The conclusion that ice loss has accelerated in recent years is independent of uncertainty in PGR effects, because, regardless of magnitude, PGR should contribute a constant rate to time series of any length. GRACE clearly detects a rate change in the most recent period, suggesting a contribution of about 0.54 mm/year to global sea level rise, well above earlier assessments (*23*). Time series are still relatively short, and an understanding of interannual variation in ice mass rates is lacking for Greenland. Without question, the extension of the GRACE mission beyond 2010, or the development of a follow-up mission, will contribute fundamentally to separating contributions of ice mass change from other geophysical signals (such as PGR) that contribute to the observations.

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Fig. S1

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Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

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The role of the adaptive immune response in controlling the growth and recurrence of human tumors has been controversial. We characterized the tumor-infiltrating immune cells in large cohorts of human colorectal cancers by gene expression profiling and in situ immunohistochemical staining. Collectively, the immunological data (the type, density, and location of immune cells within the tumor samples) were found to be a better predictor of patient survival than the histopathological methods currently used to stage colorectal cancer. The results were validated in two additional patient populations. These data support the hypothesis that the adaptive immune response influences the behavior of human tumors. In situ analysis of tumor-infiltrating immune cells may therefore be a valuable prognostic tool in the treatment of colorectal cancer and possibly other malignancies.

Tumors in mice and humans often contain infiltrates of immune cells. Experiments with immune-deficient mice have provided data supporting the role of adaptive immunity in cancer immunosurveillance (*1*–*4*). Tumor cells can express antigens and become targets for a T cell–mediated adaptive immune response (*5*, *6*). The differentiation of naïve CD4$^+$ T cells into T helper type 1 (T_H1) cells producing interferon gamma (IFN-γ) promotes CD8 T cell–mediated adaptive immunity (*7*). In mice, immune cells appear to prevent the development of tumors and inhibit tumor progression (*1*, *3*, *4*). Anti-tumor immunity also leads to immunoediting, a process favoring the eventual outgrowth of tumor cells with reduced immunogenicity (*3*).

The role of immune cells in human neoplasia is less clear (*8*). Immune cells can release inflammatory mediators with proangiogenic and prometastatic effects (*9*–*14*). Tumor-infiltrating lymphocytes in melanoma (*15*), colorectal cancers (CRCs) (*16*–*18*), and ovarian cancers (*19*, *20*) have been shown to inhibit tumor growth and are associated with improved prognoses. After antigen stimulation, a small population of antigen-specific memory T cells remains in the tissues (*21*). We recently showed that human CRCs with a high density of infiltrating memory and effector memory T cells were less likely to disseminate to lymphovascular and perineural structures and to regional lymph nodes (*22*). Using the same cohort of patients, we investigated the relationship between the type, density, and location of immune cells within tumors and the clinical outcome of the patients.

To this end, we conducted genomic and in situ immunostaining analyses on tumors from 75 and 415 patients, respectively (table S1). The data were entered into a dedicated Tumoral MicroEnvironment Database (TME.db; access available upon request). We used quantitative real-time polymerase chain reaction to evaluate the expression levels of genes related to inflammation, T_H1 adaptive immunity, and immunosuppression. These genes showed variable expression patterns in the 75 tumors studied (fig. S1). Correlation analyses performed between all genes showed 39 highly significant combinations ($P < 0.0001$) (fig. S1 and table S2). We identified a dominant cluster of co-modulated genes for T_H1 adaptive immunity [genes encoding T-box transcription factor 21, interferon regulatory factor 1, IFN-γ, CD3-ζ, CD8, granulysin, and granzyme B (GZMB)] (Fig. 1A). A hierarchical tree structure classifying the patients according to the expression levels of genes from this cluster revealed an inverse correlation between the expression of these genes and tumor recurrence (P value comparing patient groups, all $P < 0.05$) (Fig. 1B). These data suggest that T_H1 adaptive immunity has a beneficial effect on clinical outcome.

We next used tissue microarrays to investigate the in situ adaptive immune response in the center of the tumor (CT) and the invasive margin (IM) of 415 CRCs. Immunostainings for total T lymphocytes (CD3), CD8 T cell effectors and their associated cytotoxic molecule (GZMB), and memory T cells (CD45RO) were quantified with the use of a dedicated image analysis workstation (Fig. 2A and figs. S2 to S4). Tumors from patients without recurrence had higher immune cell densities (CD3, CD8, GZMB, and CD45RO) within each tumor region (CT and IM), than did those from patients whose tumors had recurred (Fig. 2B). In each tumor region (CT and IM) and for each marker (CD3, CD8, GZMB, and CD45RO), there was a statistically significant correlation between immune cell density and patient outcome for a large range of cutoff values (fig. S5). In particular, using the cutoff that yielded the minimum P value for disease-free survival, the densities of $CD3^+$, $CD8^+$, $GZMB^+$, and $CD45RO^+$ cells in each tumor region (CT and IM) allowed the stratification of patients into groups with different disease-free survival rates [P values corrected after (*23*), ranging from 1.0×10^{-2} to 4.8×10^{-6}] and overall survival rates (P values ranging from 5.5×10^{-3} to 7.9×10^{-8}) (Fig. 2C and tables S3 and S4). Reanalyses of the data using 100 repetitions of twofold cross-validations after (*24*) (tables S3 and S4) or setting the cutoff at the median of the data sets (tables S5 and S6) provided concordant results as to the prognostic value of each immune parameter.

We investigated whether the combined analysis of tumor regions could improve the prediction of patient survival. For all the markers of adaptive immunity (CD3, CD8, GZMB, and CD45RO), the combined analysis of CT plus IM regions [high density in both regions (HiHi) versus low density in both regions (LoLo)] increased the accuracy of prediction of disease-free and overall survival time for the different patient groups, as compared to single-region analysis (Hi versus Lo) (Fig. 2, D to F; figs. S6 and S7; and tables S3 to S6). Data were also analyzed using twofold cross-validation after

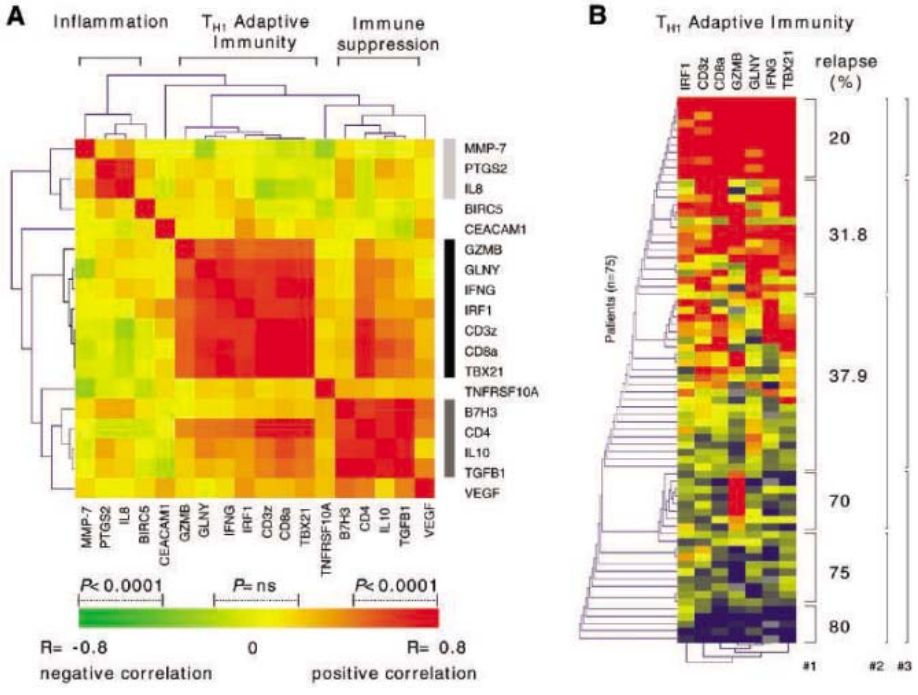

Fig. 1. (**A**) Correlation analyses performed between the 18 immunogenes were uploaded into the Genesis clustering program (*28*–*30*). A correlation matrix followed by unsupervised hierarchical clustering (Pearson uncentered algorithm) is represented from $R = -0.8$ negative correlation (green) to $R = 0.8$ positive correlation (red). For all correlations with $0.4 < R < 0.9$, $P < 0.05$ (table S1). The correlation matrix reveals a dominant cluster of co-modulated genes for T_H1 adaptive immunity and two clusters of genes encoding mediators of inflammation and immunosuppression. (**B**) Hierarchical tree structure classifying the 75 patients according to the mRNA levels of the seven genes from the T_H1 adaptive cluster, from maximal (red) to minimal (blue) expression levels. The percentage of patients with tumor recurrence (relapse) is indicated. Patients with a homogeneous increased expression of genes for T_H1 adaptive immunity had the best prognosis. Log-rank tests comparing the disease-free survival times between patient groups reached statistical significance ($P < 0.05$ for numbers 1, 2, and 3). In contrast, expression levels of inflammatory and immunosuppressive genes showed no correlation with tumor recurrence. MMP-7, matrix metalloproteinase 7; PTSG2, prostaglandin-endoperoxide synthase 2; IL8, interleukin-8; BIRC5, baculoviral IAP repeat–containing 5 (survivin); CEACAM1, carcinoembryonic antigen–related cell adhesion molecule 1; GLNY, granulysin; IRF1, interferon regulatory factor 1; TBX, T-box 21 (TBET); TNFRSF10A, tumor necrosis factor receptor superfamily, member 10a (TRAILR1); TGFB1, transforming growth factor–β 1; VEGF, vascular endothelial growth factor.

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(*24*) (100 repetitions for each marker), showing highly significant differences (tables S3 and S4). $CD3_{CT}/CD3_{IM}$ density was associated with the smallest *P* values for disease-free and overall survival analyses ($P = 7.6 \times 10^{-8}$ and $P = 4.0 \times 10^{-7}$, respectively) (tables S3 and S4). To confirm these results, we analyzed an additional cohort of patients who were different from those in the first series and a third cohort of CRC patients from another hospital. For each cohort, we determined the median cutoff values for $CD3_{CT}/CD3_{IM}$ density (50% of patients with a high density and 50% of patients with a low density). The two independent cohorts (Fig. 2, E and F) confirmed the data obtained on the first series (Fig. 2D). All statistical analyses were also performed for the subgroup of patients without concomitant distant metastasis [Union Internationale Centre le Cancer–Tumor Node Metastasis (UICC-TNM) cancer stages I, II, and III]. Significant *P* values were observed for $CD3_{CT}/CD3_{IM}$, $CD8_{CT}/CD8_{IM}$, and $CD45RO_{CT}/CD45RO_{IM}$ densities for predicting disease-free survival and overall survival (figs. S8 and S9 and tables S7 to S10).

We determined whether these immune criteria could discriminate patient outcome at each step of cancer progression. Patients were stratified according to the UICC-TNM classification (*25*) (Fig. 3A). A strong in situ immune reaction in both tumor regions correlated with a favorable prognosis regardless of the local extent of the tumor and of invasion of regional lymph nodes (stages I, II, and III). Conversely, a weak in situ immune reaction in both tumor regions correlated with a poor prognosis even in patients with minimal tumor invasion (stage I) (Fig. 3B). We recently demonstrated the importance of the density of $CD45RO^+$ memory T cells in limiting the tumor dissemination of CRCs (*22*). We found that patients with low densities of $CD3^+$ cells and $CD45RO^+$ memory T cells in both tumor regions (CT and IM) had a very poor prognosis, similar to that of patients with concomitant distant metastasis

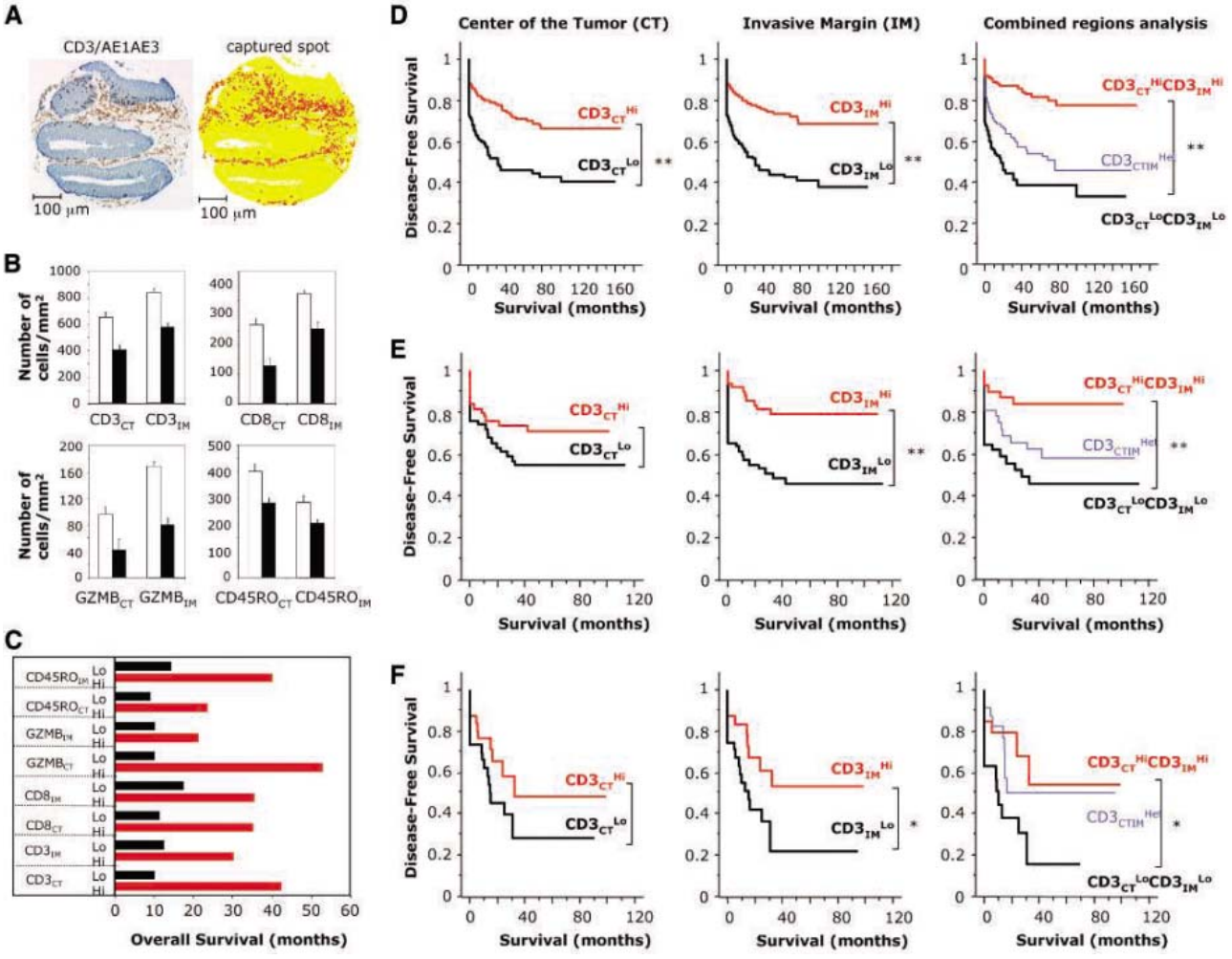

Fig. 2. (**A**) (Left) A representative example of CD3 immunostaining of a CRC tissue microarray (top). $CD3^+$ T cells (brown) and tumor cells (blue) are shown. (Right) Digital image analyzed with the image software SpotBrowser, with tissue represented in yellow and $CD3^+$ cells represented in red. The densities of adaptive immune cells ($CD3^+$, $CD8^+$, $GZMB^+$, and $CD45RO^+$ cells) were recorded as the number of positive cells per unit of tissue surface area. (**B**) Comparison of the mean (±SE) of immune cell densities in the CT and IM from patients with tumor recurrence (black bars) or without tumor recurrence recurrence (white bars). (**C**) Overall survival time for all patients, accounting for censoring (75th percentile), with high densities (red bars) or low densities (black bars) of adaptive immune cells in each tumor region (CT or IM). (**D** to **F**) Three independent cohorts of CRC patients were analyzed in a blinded manner for $CD3_{CT}/CD3_{IM}$ patterns [(D), $n = 415$; (E), $n = 119$; (F), $n = 69$ patients]. Kaplan-Meier curves illustrate the duration of disease-free survival according to the $CD3^+$ cell density in a single tumor region in the CT (left panels) or IM (middle panels) and in both tumor regions (right panels). In each cohort, for each tumor region, high (Hi) and low (Lo) CD3 densities were plotted according to the cutoff value of $CD3^+$ cell density defined at the median of the cohort (50% of patients with high cell density and 50% of patients with low cell density). In single-region analysis (left and middle panels), red lines indicate $CD3^{Hi}$ and black lines indicate $CD3^{Lo}$. In combined analysis (right panels), red lines indicate $CD3_{CT}^{Hi}CD3_{IM}^{Hi}$, black lines indicate $CD3_{CT}^{Lo}CD3_{IM}^{Lo}$, and blue lines indicate heterogeneous CD3 densities with $CD3_{CT}^{Lo}$ plus $CD3_{IM}^{Hi}$ or $CD3_{CT}^{Hi}$ plus $CD3_{IM}^{Lo}$ ($CD3_{CTIM}^{Het}$).

(stage IV) (Fig. 3C). In multivariate analysis, after adjusting for tumor invasion (T stage), tumor differentiation, and lymph node invasion (N stage), $CD3_{CT}/CD3_{IM}$ density (HiHi, Heterogeneous, and LoLo) remained an independent prognostic factor, with the highest hazard ratio (HR) and the smallest P value in disease-free survival analysis [HR = 2.379; $P = 1.4 \times 10^{-6}$, corrected after (*26*)] (table S11). $CD3_{CT}/CD3_{IM}$ density was the only independent parameter associated with overall survival (HR = 1.89; $P = 1.2 \times 10^{-5}$) (table S12). The histopathological parameters were no longer associated with disease-free and overall survival in patients with coordinated high or low densities of the immune markers in both tumor regions (HiHi versus LoLo) (tables S11 and S12).

Our results suggest that once human CRCs become clinically detectable, the adaptive immune response plays a role in preventing tumor recurrence. Despite immunoediting (*2*), the beneficial effect of the adaptive immunity may persist throughout tumor progression (stages II and III). Intratumoral T cells could modify tumor stroma or tumor cells in ways that attenuate the metastatic potential of tumor cells. We found a positive correlation between the presence of markers for T_H1 polarization and of cytotoxic and memory T cells and a low incidence of tumor recurrence. This argues for immune-mediated rejection of persistent tumor cells after surgery. We hypothesize that the trafficking properties and long-lasting anti-tumor capacity of memory T cells (*27*) play a central role in the control of tumor recurrence.

The type, density, and location of immune cells in CRCs had a prognostic value that was superior to and independent of those of the UICC-TNM classification (*25*). This suggests that time to recurrence and overall survival time are governed in large part by the state of the local adaptive immune response. The immunological criteria that we have used may lead to revision of the current indicators of clinical outcome and may help identify the high-risk patients who would benefit most from adjuvant therapy. Finally, this approach may be useful for the investigation of other tumor types.

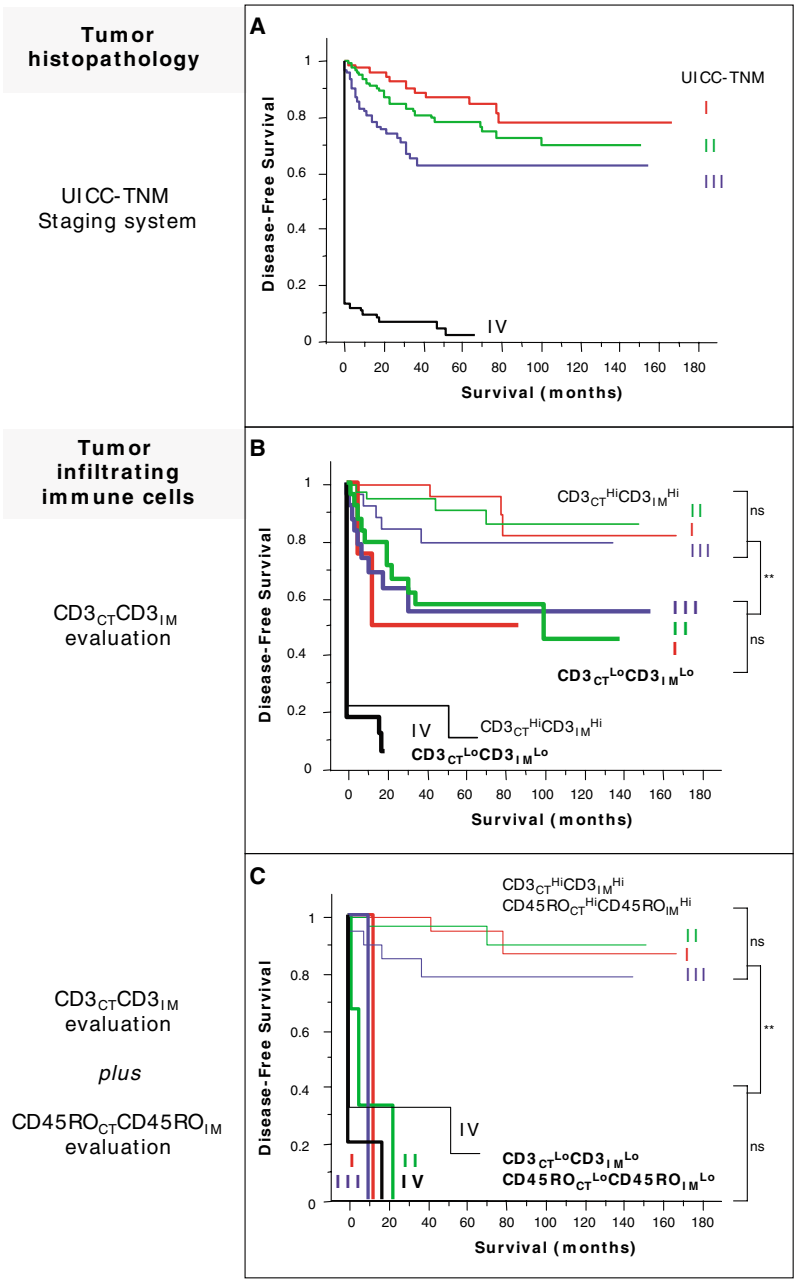

Fig. 3. (**A**) Kaplan-Meier curves illustrate the duration of disease-free survival according to the UICC-TNM stages [stage I, red line (n = 75 patients); stage II, green line (n = 137); stage III, blue line (n = 99), and stage IV, black line (n = 95)] in patients with CRCs. (**B**) Kaplan-Meier curves illustrate the duration of disease-free survival according to the UICC-TNM stages [as in (A)] and according to the density of $CD3^+$ cells in combined tumor regions ($CD3_{CT}^{Lo}CD3_{IM}^{Lo}$, thick lines, n = 93 patients; $CD3_{CT}^{Hi}CD3_{IM}^{Hi}$, thin lines, n = 109). The subgroup of patients that did not appear to have a coordinated in situ immune reaction in tumor regions (Hi/Lo or Lo/Hi for $CD3^+$ cell densities) presented similar Kaplan-Meier curves as the entire cohort (fig. S10).(**C**) Kaplan-Meier curves illustrate the duration of disease-free survival according to the UICC-TNM stages and to the density of $CD3^+$ and $CD45RO^+$ cells in combined tumor regions ($CD3_{CT}^{Lo}CD3_{IM}^{Lo}$ plus $CD45RO_{CT}^{Lo}CD45RO_{IM}^{Lo}$, thick lines, n = 16 patients; $CD3_{CT}^{Hi}CD3_{IM}^{Hi}$ plus $CD45RO_{CT}^{Hi}CD45RO_{IM}^{Hi}$, thin lines, n = 88). Cutoff values were 250, 640, 60, and 190 for $CD3_{CT}$, $CD3_{IM}$, $CD45RO_{CT}$, and $CD45RO_{IM}$, respectively. In (B) and (C), log-rank statistical test, ** $P < 10^{-4}$; ns, not significant.

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Volatile Chemical Cues Guide Host Location and Host Selection by Parasitic Plants

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The importance of plant volatiles in mediating interactions between plant species is much debated. Here, we demonstrate that the parasitic plant *Cuscuta pentagona* (dodder) uses volatile cues for host location. *Cuscuta pentagona* seedlings exhibit directed growth toward nearby tomato plants (*Lycopersicon esculentum*) and toward extracted tomato-plant volatiles presented in the absence of other cues. Impatiens (*Impatiens wallerana*) and wheat plants (*Triticum aestivum*) also elicit directed growth. Moreover, seedlings can distinguish tomato and wheat volatiles and preferentially grow toward the former. Several individual compounds from tomato and wheat elicit directed growth by *C. pentagona*, whereas one compound from wheat is repellent. These findings provide compelling evidence that volatiles mediate important ecological interactions among plant species.

Plant volatiles serve as important foraging cues for both insect herbivores and their natural enemies and can convey complex information regarding plant location, identity, and condition (*1*–*5*). It has been suggested that volatiles may have similar importance for interactions among plants, but such claims have remained controversial (*6*–*13*) and where plant-plant volatile effects have been demonstrated, their ecological importance remains unclear (*6*–*9*). Previous work on volatile-mediated interactions among plant species has dealt with the role of volatiles induced by herbivory or other environmental stressors in initiating defensive responses in neighboring plants (*7*, *14*–*19*). Parasitic plants, which to survive must rapidly locate and attach to other plants, provide an alternative system in which host-plant volatiles might be expected to play an important role.

Parasitic plants are important components of both natural and agricultural ecosystems and have considerable influence on the structure and dynamics of the communities they inhabit (*20*, *21*). Yet, little is known about the ecology of interactions between parasitic plants and their hosts. Like insect herbivores, parasitic plants exhibit various "foraging" patterns (*22*–*25*) and are capable of "selecting" among potential hosts (*22*–*25*), but the mechanisms involved in host location and discrimination are not well understood.

Flowering plants in the genus *Cuscuta* are obligate parasites with little photosynthetic capability; they obtain nutrients by attaching to aboveground shoots of other plants (*26*) (Fig. 1). *Cuscuta* spp. are important agricultural pests, included on the U.S. Department of Agriculture's *Top Ten Weeds List*, and can be difficult to control without also impacting host plants (*27*). Seeds of *Cuscuta* spp. contain minimal energy reserves, allowing growth of only several centimeters, and upon germination, the rootless seedlings must locate and attach to a suitable host within a few days (*26*). In some parasitic plants, contact with chemical cues secreted from host-plant roots is required for germination (*28*, *29*), but *Cuscuta* spp. have no specialized germination requirements and must depend on seedling "foraging" for host-plant location (*26*) (fig. S1). After germination, *C. pentagona* seedlings exhibit a rotational growth habit (circumnutation) until contacting a host (*26*) (movie S1). Host secondary metabolites are known to influence the belowground growth of parasitic plants that attach to host roots (*28*, *29*), and host-derived chemicals also induce haustorial development by these parasites (*30*). However, the role of host-derived compounds in aboveground host location by *Cuscuta* spp. has not previously been determined.

In this study, we explored host finding by seedlings of *C. pentagona*. First, we examined whether *C. pentagona* seedlings exhibit directed growth toward host plants (potted 20-day-old tomato seedlings). The basal end of a *C. pentagona* seedling was inserted into a water vial placed at the center of a dry filter-

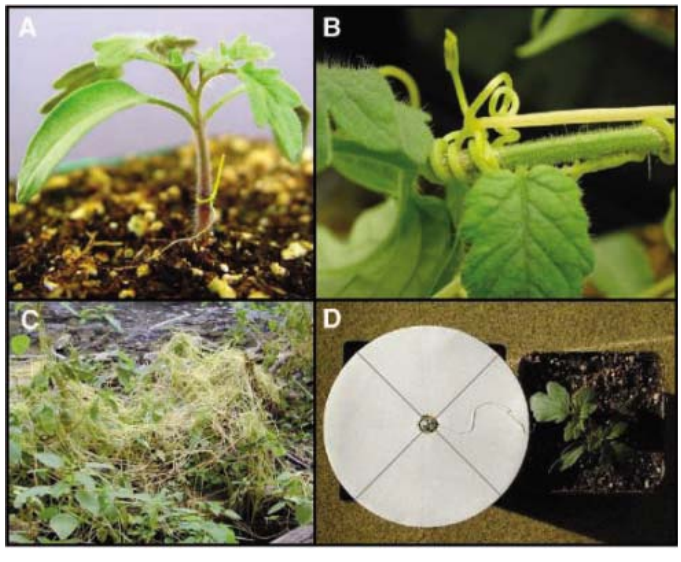

Fig. 1. Parasitic plants in the genus *Cuscuta*. (**A**) *C. pentagona* seedling attaching to a tomato plant. (**B**) Vines of *C. pentagona* coiled around the petiole of a tomato leaf. (**C**) Growth habit of *Cuscuta*. (**D**) *C. pentagona* seedling growing toward a tomato plant across a filter-paper disc.

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paper disc. A host plant was placed near the edge of the disc (Fig. 1D), and the seedling was allowed to "forage" for 4 days. Seedlings' growth across the discs was recorded by tracing their position on the filter paper (Figs. 1D and 2A). Our initial assay determined whether plants grew into the semicircle (disc half) adjacent to the target plant or into the semicircle opposite the target. This assay yielded statistically significant results (80% grew toward the host plant) (Table 1), indicating that directed growth does occur. Visual observation of the recorded growth patterns further suggested that a large proportion of plants grew more or less directly toward the target plant. To quantify this impression, we divided the disc into four quadrants (Fig. 2A) and used chi-square analysis to compare expected and observed numbers of plants growing into each. More seedlings than expected by chance grew into the quadrant nearest the target, whereas significantly fewer grew into the quadrant directly opposite the target (Table 1).

These results provide strong evidence for directed growth by *C. pentagona* seedlings toward host plants but do not establish the cues responsible for eliciting this growth. Because we suspected a role for host-plant volatiles, we used the experimental design described above to test seedling growth responses to control targets designed to mimic possible alternative cues. Targets included pots of moist soil without plants, artificial tomato seedlings, and vials of green- or red-colored water. None of these control targets elicited a growth response from *C. pentagona* seedlings (Table 1). However, these controls provided at best a crude representation of the cues available from actual host plants, and the lack of response to these targets does not conclusively eliminate a possible role for shading or other light cues in host location. The moist soil control does indicate that the cues involved in host location, volatile or otherwise, are derived from the host plants themselves (Fig. 2A and Table 1).

To demonstrate more firmly a role for volatile cues in host location, we placed *C. pentagona* seedlings, arranged on filter-paper discs as before, in a small open-air enclosure linked to two enclosed target chambers by short lengths of black polyvinyl chloride pipe, each with an intervening 90° bend (Fig. 2B). Four potted 20-day-old tomato seedlings were placed in one of the target chambers and four artificial tomato plants in pots of moist soil in the other. This configuration was designed to permit volatile transmission while blocking most light cues. Previous studies testing plant response to volatiles have been criticized for using airtight chambers that produce elevated volatile concentrations and may influence the physiological status of plants (*6*–*8*, *13*). Our open system avoided such problems. Multiple plants were used to increase volatile concentrations, because the design of this experiment necessitated placing host plants unrealistically far away from the *C. pentagona* seedlings (i.e.,

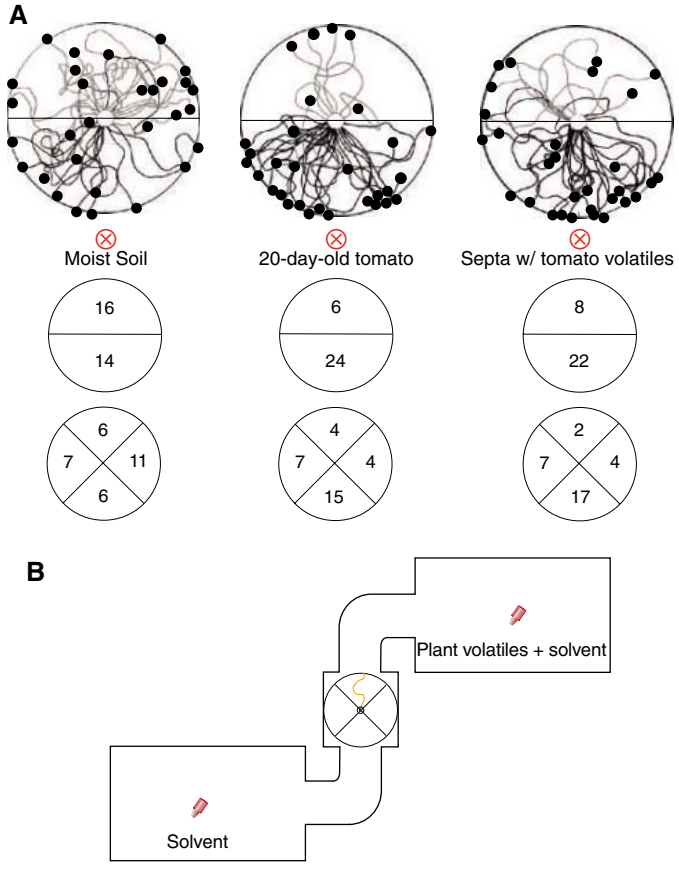

Fig. 2. Foraging by *Cuscuta pentagona* seedlings. (**A**) Summary of *C. pentagona* seedling growth responses to a pot containing moist soil, a nearby 20-day-old tomato plant, and tomato volatiles released from rubber septa. The position of the target is indicated by a circled X. The final position of the apex of each seedling is highlighted with a solid black circle. The numbers of seedlings growing into each disc half and quadrant are summarized in the smaller circles below each disc. (**B**) Experimental setup for the release of plant volatiles while blocking light cues.

Table 1. Foraging of *Cuscuta pentagona* seedlings on filter paper discs to various targets.

Exp	Target	Seedlings choosing disc half with or without targets					Seedlings choosing quadrants (direction relative to target)				
		No. with target	No. without target	% with target	% without target	χ^2 (*P* value)	A (away)	B (side)	C (side)	D (toward)	χ^2 (*P* value)
1	10-day-old plants	23	7	77	23	8.53 (0.004)	4	5	4	17	16.1 (0.001)
2	20-day-old plants	24	6	80	20	10.8 (0.001)	4	4	7	15	10.8 (0.013)
3	Red glass	14	16	47	53	0.13 (0.715)	11	8	6	5	2.80 (0.423)
4	Green glass	12	18	40	60	1.20 (0.273)	13	9	3	5	7.87 (0.052)
5	Artificial plant	12	18	40	60	1.20 (0.273)	8	8	9	5	1.20 (0.753)
6	Moist soil	14	16	47	53	0.13 (0.715)	6	7	11	6	2.27 (0.519)
7	20-day-old plants*	23	7	77	23	8.53 (0.004)	4	6	4	16	13.2 (0.004)
8	Volatile extracts*	22	8	73	27	6.53 (0.011)	2	7	4	17	17.7 (< 0.001)

*Target tested in experimental enclosure.

Table 2. Pair-wise test using logistic regression to contrast different target treatments. χ^2 (*P* value).

Exp.	1	2	3	4	5	6	7	8
1	–	0.098 (0.754)*	**5.47 (0.019)‡**	**7.82 (0.005)‡**	**7.82 (0.005)‡**	**5.47 (0.019)‡**	0.0 (1.0)*	0.089 (0.766)*
2			**6.75 (0.009)‡**	**9.25 (0.002)‡**	**9.25 (0.002)‡**	**6.75 (0.009)‡**	0.098 (0.754)*	0.371 (0.543)*
3				0.271 (0.603)†	0.271 (0.603)†	0.0 (1.0)†	**5.47 (0.019)‡**	**4.31 (0.038)‡**
4					0.0 (1.0)†	0.271 (0.603)†	**7.82 (0.005)‡**	**6.49 (0.011)‡**
5						0.271 (0.603)†	**7.82 (0.005)‡**	**6.49 (0.011)‡**
6							**5.47 (0.019)‡**	**4.31 (0.038)‡**
7								0.089 (0.766)*
8								–

*Group A (Exps. 1, 2, 7, and 8) †Group B (Exps. 3, 4, 5, and 6) ‡Contrast tests between groups A and B

Table 3. Average volatiles released by 20-day-old tomato plants and by rubber septa treated with tomato volatiles.

Compound	Volatiles released (ng/24hours ± SEM)	
	Four 20-day-old tomato plants	Rubber septum treated with tomato volatiles
α-Pinene	83.8 ± 13.9	10.8 ± 3.9
β-Myrcene	93.5 ± 6.2	44.5 ± 8.8
2-Carene	1131.6 ± 173.4	448.3 ± 89.6
p-Cymene	53.6 ± 13.1	50.4 ± 13.9
β-Phellandrene	2843.9 ± 395.8	1457.6 ± 367.7
Limonene	602.7 ± 64.6	346.2 ± 85.4
(*E*,*E*)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene	376.9 ± 141.8	176.5 ± 35.8
Unidentified monoterpene	138.9 ± 18.2	52.9 ± 14.2

farther than a seedling could grow before exhausting its energy reserves). We observed a directed growth response similar to that in our first experiment. Significantly more *C. pentagona* seedlings grew toward the target chamber containing host seedlings than toward the chamber containing artificial plants (77% grew toward host plants) (Table 1). This response was statistically indistinguishable from that to a single tomato plant in a completely open system (Table 2). Dividing the discs into quadrants again revealed more seedlings than would be expected by chance growing more or less directly toward the target and fewer growing directly away from the target (Table 1).

This result strongly suggests a role for host-plant volatiles in host location by *C. pentagona* seedlings; however, we cannot rule out the possibility that this experimental design still allows the transmission of some alternative cues. To establish conclusively a role for volatile cues, we used the same experimental design to test seedling growth responses to extracted host volatiles experimentally released from rubber septa in the absence of any other plant-derived cues. Volatiles were collected from four 20-day-old tomato plants onto SuperQ (Alltech Associates, Deerfield, IL) adsorbent filters. Extracts from these filters were then released from a rubber septum placed in one of the target chambers (Fig. 2B). A septum containing solvent alone was placed in the other chamber. Gas chromatographic analysis revealed that undamaged tomato seedlings released eight major volatile compounds [α-pinene, β-myrcene, 2-carene, *p*-cymene, β-phellandrene, limonene, (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, and one unidentified monoterpene] and that rubber septa treated with tomato volatile extracts released the same compounds in about the same proportions as intact plants but in lesser amounts (Table 3). We observed a growth response to extracted volatiles similar to that observed in response to whole plants: Significantly more *C. pentagona* seedlings grew toward the target chamber containing the septum with extracted host volatiles than toward the chamber containing the septum with solvent alone (73% grew toward host-plant volatiles) (Table 1 and Fig. 2). Once again, dividing the discs into quadrants revealed an excess of seedlings growing more or less directly toward the target and fewer than would be expected by chance growing directly away from the target (Table 1 and Fig. 2A).

A pairwise comparison using logistic regression showed no significant difference in seedling responses to the three tomato volatile treatments (a single tomato plant, four tomato plants in the experimental enclosure, or extracted volatiles) but did show significant differences between the tomato volatile treatments and all other targets (Table 2), providing further confirmation of a role for host-plant volatiles in foraging by *C. pentagona* seedlings. These results demonstrate decisively that *C. pentagona* seedlings exhibit directed growth toward volatile compounds derived from tomato plants and strongly suggest that this is an adaptive mechanism for host location.

In a subsequent experiment, we found that *C. pentagona* seedlings also exhibited directed growth toward nearby cultivated *Impatiens wallerana* 'Dazzler' (disc half: $\chi^2 = 6.53$, $P = 0.01$; quadrant: $\chi^2 = 10.27$, $P = 0.01$, $n = 30$). Wheat plants (*Triticum aestivum* 'McNeal'), an unsuitable host on which *C. pentagona* does not survive (*26*), elicited a growth response that was statistically marginal ($\chi^2 = 3.33$, $P = 0.06$, $n = 30$); however, a small increase in sample size yielded a significant result (disc half: $\chi^2 = 5.57$, $P = 0.01$; quadrant: $\chi^2 = 8.09$, $P = 0.04$, $n = 34$). These results suggest that *C. pentagona*'s host-location mechanism operates across a wide range of plant species.

Having established the role of volatiles in host-plant location by *C. pentagona*, we examined whether *C. pentagona* seedlings were also able to distinguish between potential hosts of differing quality. When *C. pentagona* seedlings were planted between tomato (host) and wheat (nonhost) seedlings and equidistant from each, they exhibited a strong and consistent growth bias toward tomato ($\chi^2 = 12.57$, $P < 0.001$, $n = 23$). This result cannot be explained by contact cues, because there were no cases in which *C. pentagona* seedlings contacted one host before attaching to the other. To confirm that this host preference was mediated by plant volatiles, we gave seedlings a choice between rubber septa treated with extracted tomato and wheat volatiles (using the setup described above for extracted tomato volatiles) (Fig. 2B). *Cuscuta pentagona* seedlings exhibited a clear preference for extracted tomato volatiles ($\chi^2 = 6.53$, $P = 0.011$, $n = 30$). This result suggests that, although *C. pentagona* may respond to a variety of plant odors, it is capable of preferentially responding to volatiles produced by its preferred hosts.

To explore the contribution of individual compounds to the attractiveness of host volatiles, we used the same assay previously described for whole plants (Fig. 1D) to examine the growth responses of *C. pentagona* seedlings to synthetic standards released from rubber septa. When we tested seven identified compounds from the tomato blend, a significant positive response was observed to

Table 4. Foraging of *Cuscuta pentagona* seedlings on filter paper discs to individual tomato (top) and wheat (bottom) volatiles released from rubber septa.

Volatile compound	Seedlings choosing disc half with or without volatile					Seedlings choosing quadrants (direction relative to volatile)				
	No. with volatile	No. without volatile	% with volatile	% without volatile	χ^2 (*P* value)	A (away)	B (side)	C (side)	D (toward)	χ^2 (*P* value)
α-Pinene	23	11	68	32	4.23 (0.039)	6	8	9	11	1.53 (0.676)
β-Myrcene	21	9	70	30	4.80 (0.029)	6	6	4	14	7.87 (0.049)
2-Carene	14	20	41	59	1.06 (0.304)	11	9	8	6	1.53 (0.676)
p-Cymene	17	13	57	43	0.53 (0.465)	5	7	9	9	1.47 (0.690)
β-Phellandrene	21	9	70	30	4.80 (0.029)	5	6	6	13	5.47 (0.141)
Limonene	16	14	53	47	0.13 (0.715)	9	6	5	10	2.27 (0.519)
TMTT*	14	16	47	53	0.13 (0.715)	8	8	5	9	1.20 (0.753)
(*Z*)-3-Hexenyl acetate	11	23	32	68	4.23 (0.039)	13	5	9	7	4.12 (0.249)
(*Z*)-3-Hexen-1-ol	15	19	44	56	0.47 (0.493)	13	6	6	9	3.88 (0.275)
(*E*)-β-Ocimene	16	14	53	47	0.13 (0.715)	3	7	10	10	4.40 (0.221)
Linalool	14	16	47	53	0.13 (0.715)	9	8	9	4	2.27 (0.519)
Decanal	22	12	65	35	2.94 (0.086)	8	7	9	10	0.59 (0.899)
Nonanal	17	17	50	50	0.00 (1.000)	6	15	6	7	6.71 (0.082)

*(*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene

β-phellandrene, β-myrcene, and, after a small increase in sample size, α-pinene (Table 4). Notably, β-myrcene is also released by wheat seedlings. Six other compounds released by 20-day-old wheat seedlings did not show a significant positive growth response (Table 4). Unexpectedly, one wheat compound, (*Z*)-3-hexenyl acetate, appeared to have a repellant effect—although this result initially was not significant ($\chi^2 = 3.33$, $P = 0.06$, $n = 30$), a small increase in sample size yielded statistical significance (Table 4). This finding suggests a possible mechanism for the observed preference for the volatile blend produced by the preferred host tomato over that produced by the nonhost wheat.

The positive growth response observed to individual compounds suggests that these compounds may be important for host location and discrimination. However, complex qualitative features of the blend may play an important role (*31*). Until the detailed mechanisms by which *C. pentagona* perceives and responds to host-plant volatiles are elucidated, it will be difficult to determine exactly how the information content of the signal is encoded in the volatile blend, because cross talk may occur between components of the blend or their effects on the receiver (*6*). Because of its parasitic life-style and the concomitant reduction in physiological complexity (e.g., the almost complete absence of photosynthesis and leaves), *C. pentagona* may provide an excellent model system for further investigation of the mechanisms by which plants perceive and respond to volatile signals.

Aboveground plant structures have previously been shown to exhibit directed growth in response to light, gravity, humidity, and physical contact (*32*). Our results demonstrate that directed growth can also be elicited by airborne chemical cues. In addition, our findings provide insight into the host-location and host-selection mechanisms used by parasitic plants, showing that host-plant volatiles play a role in this system similar to that previously described for foraging insect herbivores (*1*) and thus revealing unexpected convergence in the host-location strategies used by disparate natural enemies of plants. Finally, our results provide an example of chemical communication between plant species that plays an important role in mediating interspecific ecological interactions. We expect these findings to have broad implications for research in a variety of fields, including chemical ecology, parasite-host interactions, and plant biology. Moreover, these results provide knowledge that may be useful in developing new tactics for controlling parasitic plants that attack agricultural crops.

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Supporting Online Material

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Structure of the Exon Junction Core Complex with a Trapped DEAD-Box ATPase Bound to RNA

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In higher eukaryotes, a multiprotein exon junction complex is deposited on spliced messenger RNAs. The complex is organized around a stable core, which serves as a binding platform for numerous factors that influence messenger RNA function. Here, we present the crystal structure of a tetrameric exon junction core complex containing the DEAD-box adenosine triphosphatase (ATPase) eukaryotic initiation factor 4AIII (eIF4AIII) bound to an ATP analog, MAGOH, Y14, a fragment of MLN51, and a polyuracil mRNA mimic. eIF4AIII interacts with the phosphate-ribose backbone of six consecutive nucleotides and prevents part of the bound RNA from being double stranded. The MAGOH and Y14 subunits lock eIF4AIII in a prehydrolysis state, and activation of the ATPase probably requires only modest conformational changes in eIF4AIII motif I.

In higher eukaryotes, the pre-mRNA splicing reaction leads to the deposition of the exon junction complex (EJC) on mature mRNAs; the EJCs are deposited at a conserved position located upstream of exon junctions and exclusively dictated by the splicing machinery (*1*). The core of the EJC is a heterotetramer that contains four proteins: eIF4AIII, MLN51, MAGOH, and Y14. The EJC constitutes a central effector of mRNA functions; it forms a stable and sequence-independent grip on mRNA and provides an anchoring point for nuclear and cytoplasmic factors participating in mRNA transport, translation, and quality control (*2*). Reconstitution of the EJC core revealed that it is an ATP-dependent complex. Its stable association with mRNA is maintained by MAGOH and Y14 through inhibition of the DEAD-box protein eIF4AIII ATPase activity (*3*). DEAD-box proteins use the energy from ATP hydrolysis to unwind double-stranded nucleic acid or rearrange RNA-protein complexes at virtually every step of the gene expression pathway (*4*–*6*). The translation initiation factor eIF4A (we refer to eIF4AI and its isoform eIF4AII collectively as eIF4A) and its close but functionally distinct homolog eIF4AIII are considered archetypal members of the DEAD-box family. By comparison to eIF4A, eight eIF4AIII specific sequence motifs (patches A to H, fig. S1) were identified (*7*).

To shed light on both the EJC core architecture and the molecular mechanism of DEAD-box proteins, we determined the crystal structure of a minimal reconstituted EJC core assembled on a poly(U) oligonucleotide mimicking the mRNA at 2.3 Å resolution (*8*) (table S1). The EJC core is an elongated complex with overall dimensions of 99 by 67 by 54 Å organized around eIF4AIII (Fig. 1A). Extensive intersubunit interactions lead to burial of 23 to 36% of their surface areas (table S2). As in VASA (*9*)—a DEAD-box ATPase that regulates translation of specific mRNAs during early development in *Drosophila*—the two domains of eIF4AIII adopt a closed conformation forming composite binding sites for the 5′-adenylyl-β-γ-imidodiphosphate (ADPNP) and the RNA. In the open conformation, found in the crystal structure of free eIF4AIII that was determined at 3.3 Å resolution (Fig. 1B), the two domains had rotated by 160° relative to each other compared with the closed conformation. For the MLN51 fragment expressed for this study (residues 137 to 283), only residues 170 to 194 and 216 to 246 could be traced, and the fragment only contains limited secondary structure (Fig. 1A). Residues 216 to 246 contact patches C, D, and E in eIF4AIII domain 1 (Fig. 1C and fig. S2), explaining why the mutation of MLN51 Tyr^{240} and Gly^{241} disrupts formation of the EJC core (*3*). Residues 170 to 194 are located at the 5′ end of the bound RNA and contact eIF4AIII domain 2 (Fig. 1C). The side chain of MLN51 Phe^{188} stacks with the base of RNA U1 (Fig. 2E), confirming that MLN51 directly contacts RNA and increases RNA binding efficiency when bound to eIF4AIII (*3*). The flexible non-conserved linker (*10*) between the two ordered MLN51 fragments allows the protein to remain associated with rather different conformations of eIF4AIII. Small angle x-ray scattering (SAXS) indicates that, both alone and in complex with the MLN51 fragment, eIF4AIII in solution adopts a conformation that resembles the open conformations of free eIF4AIII or eIF4AI (*11*) more than the closed conformation (fig. S2, E and F).

The structure of the MAGOH-Y14 heterodimer located at the 5′ pole of the EJC (oriented relative to the bound RNA; Fig. 1A) displays only subtle conformational changes relative to the free heterodimer (*12*–*14*). Residues from the two C-terminal helices and one end of the large β sheet in MAGOH contact eIF4AIII domain 2 (Figs. 1A and 2, A to D). In the free eIF4AIII, the linker (Lys^{242} to Gly^{250}) between the two eIF4AIII domains is buried between motifs V and VI in eIF4AIII domain 2 (fig. S3). Within the EJC, conserved residues of the linker form salt bridges or hydrogen bonds with Tyr^{34}, Lys^{48}, and Lys^{142} in MAGOH (Fig. 2D). The second loop in the MAGOH β sheet contacts both the two eIF4AIII domains and their linker (fig. S2C). Two loops in the MAGOH β sheet interact with residues 190 to 194 in MLN51 (Fig. 2, A and B, and fig. S2D), explaining why the mutation of Lys^{16} to Phe^{17} in MAGOH disrupts the assembly of the EJC (*15*). The C-terminal extremity of MAGOH points toward the eIF4AIII ATP binding site in the EJC. The only Y14-eIF4AIII contact is a salt bridge between Y14 Arg^{108} and eIF4AIII Asp^{401} (fig. S3C). Both residues are strictly conserved, and the double mutation (Leu^{106}→Glu^{106} and Arg^{108}→Glu^{108}) in Y14 prevents association of MAGOH-Y14 with the rest of the EJC (*15*).

Six uracil nucleotides of RNA are tightly bound to the EJC (Fig. 2E and fig. S4) in agreement with the seven to nine nucleotides that are protected from ribonuclease (RNase) treatment (*1*, *3*). Nucleotides U1 and U2 at the RNA 5′ end are in contact with domain 2 of eIF4AIII, U3 interacts with both domains, and U4 to U6 bind domain 1 of eIF4AIII. The conformation of the bound RNA and the protein-RNA interactions in the EJC are notably similar to those observed in VASA (fig. S4). Residues in the helicase motifs Ia, Ib, IV, and V in eIF4AIII exclusively contact the sugar-phosphate backbone of all the RNA residues (Fig. 2E). Similar to VASA (*9*), residues outside these motifs also interact with the RNA. All RNA phosphates are recognized by hydrogen bonds or salt bridges, and four of the six 2'OH groups are recognized by eIF4AIII (Fig. 2E), which explains how eIF4AIII can bind RNA in a sequence-independent manner (*1*). The conformation of nucleotides U1 to U4 is very close to that of one strand in double-stranded A-form RNA (A-RNA) (fig. S4). Between U4 and U5, there is a sharp kink in the ribose-phosphate backbone induced by eIF4AIII motif Ib. The conformation of nucleotides U5 and U6 also resembles that of A-RNA, and these nucleotides could potentially be part of double-stranded RNA (dsRNA), as could nucleotides upstream of U1. Based on the structure of VASA, it was suggested that the four nucleotides 5′ of the RNA kink (U1 to U4 in the EJC) could be part of dsRNA if minor structural rearrangements took place (*9*). Our modeling of a double-stranded A-RNA into both the EJC and VASA structures shows that binding of A-form dsRNA would require a large rearrangement in

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the highly conserved residues 192 to 200 (the post-II region; fig. S4). Therefore, in the closed conformation of eIF4AIII, both motif Ib and the post-II region constitute macromolecular wedges forcing strand separation within dsRNA, but nucleotides both upstream and downstream of U1 to U4 may still be base paired. These features explain how eIF4A can bind to dsRNA (*16*) and suggest a partial mechanism for unwinding and messenger ribonucleoprotein particle (mRNP) remodeling in the DEAD-box helicases. The requirement of 12 to 18 nucleotides for efficient eIF4A ATPase activation (*17*), which contrasts the 6 to 7 nucleotides found in the EJC and VASA structures (*9*), can be explained by assuming that initial RNA binding occurs to the open conformations of eIF4A (*11*) and eIF4AIII, which have been observed by crystallography and SAXS.

As for the RNA recognition, almost identical binding sites for ATP are observed in VASA (*9*) and the EJC (Fig. 3 and fig. S5). The ADPNP is sandwiched between domains 1 and 2 of eIF4AIII, interacting with the conserved motifs F, Q, I, II, V, and VI. The base is stacked between Phe^{58} and Tyr^{371}. MAGOH interlocks several of the eIF4AIII sequence motifs at the ATP site (Figs. 2C and 3A). MAGOH Ile^{146} stabilizes the position of eIF4AIII Tyr^{371} after motif VI, and MAGOH Ile^{143} makes a hydrogen bond through a water molecule with Gln^{83} in eIF4AIII motif I. Finally, MAGOH Pro^{145} forms a hydrogen bond with Ala^{63} in the Q motif of eIF4AIII. In eIF4AIII motif III, Thr^{220} links Asp^{190} and His^{363} from motifs II and VI, respectively, explaining the importance of eIF4A motif III and Asp^{190} in linking ATP hydrolysis and RNA unwinding (*18*). In addition, Asp^{190} interacts electrostatically with Arg^{339} in motif V. This interaction is probably required for positioning motif V, which links the RNA and ATP binding sites. This motif undergoes a large conformational change as a consequence of the EJC formation, where it interacts with RNA, ATP, motif II, and motif VI, whereas in the free eIF4AIII it interacts with motif VI and the linker between eIF4AIII domains 1 and 2 (fig. S3). These differences suggest motif V as an additional important mediator of cooperativity between ATP and RNA binding in eIF4AIII (*19*) and eIF4A (*20*).

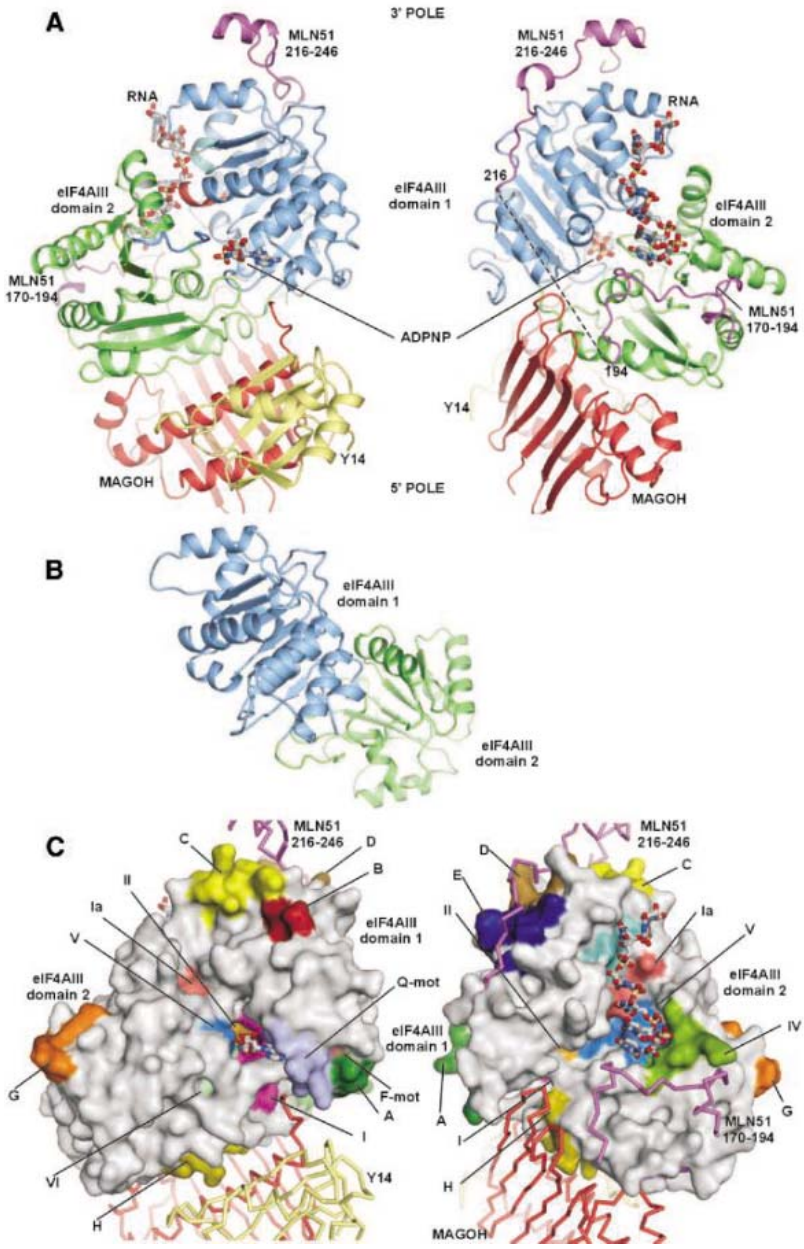

Fig. 1. Structures of the EJC and free eIF4AIII. (**A**) The EJC viewed from the ATP side (left) and the RNA side (right) with domains 1 and 2 of eIF4AIII colored blue and green, respectively. MLN51 is shown in purple, Y14 in yellow, and MAGOH in red. The dotted line connects the two ordered fragments of MLN51. (**B**) The open conformation of eIF4AIII with domain 1 in the same orientation as in the left panel of (A). (**C**) Surface representation of eIF4AIII, in which the three other subunits are shown as Cα skeletons. Conserved DEAD-box motifs (*4*) and eIF4AIII specific patches (*7*) (fig. S1) are mapped.

The VASA helicase core structure represents an active state of the DEAD-box ATPase (*9*). Although the use of ADPNP in both the EJC and the VASA structures may cause subtle differences to the experimentally inaccessible ATP-bound states, by comparing the two otherwise almost identical structures of DEAD-box ATPases bound to RNA and ADPNP, we can examine the molecular basis for the inhibition of the eIF4AIII ATPase activity by MAGOH-Y14. The γ-phosphate is shielded from bulk water in the EJC with three water molecules trapped in its vicinity. One of these (w_c, Fig. 3) is a likely candidate for a nucleophilic water molecule attacking the γ-phosphate during ATP hydrolysis. A slight reorientation of the γ-phosphate between the EJC and the VASA structures probably explains the different states of the two ATPases. In the EJC, the distance between the catalytic water and the γ-phosphorus is 3.52 Å, and the inline attack angle between w_c, the phosphorus atom, and the nitrogen linking the γ- and β-phosphates is 160°, whereas the corresponding values in VASA are 3.25 Å and 176°. Thus, the geometry between the nucleophilic centers is more favorable for attack in VASA (Fig. 3D). The suggested importance of the γ-phosphate reorientation is supported by an unexpected homology to the mitochondrial F1-ATPase (fig. S5), which also suggests that a second water molecule (w_r, Fig. 3) functions in a proton relay during ATP hydrolysis.

The γ-phosphate is coordinated by motifs I and VI, and given that there are no obvious differences in motif VI between the structures of EJC and VASA, its reorientation between the two structures must be caused by differences in motif I. The most notable change occurs at eIF4AIII Thr^{89}. In the EJC, its side chain coordinates the Mg^{2+} ion, but with a distorted octahedral geometry. In VASA, after a 1.6 Å

shift, it is no longer a Mg^{2+} ligand; instead, a water molecule (w_t) coordinates the ion in a regular octahedral geometry (Fig. 3C). Considering the otherwise almost perfect agreement between the two structures, this shift most likely forms a major component in the transition from the inhibited ATPase in the EJC to the active ATPase in VASA. The absence of the threonine in the Mg^{2+} coordination sphere in VASA is unusual, but the imperfect coordination of Mg^{2+} in the EJC and the demonstrated ATPase and unwinding activity of the VASA fragment (*9*) strongly suggests its physiological relevance. In addition, the difference in the backbone around Ser^{84} in eIF4AIII and Thr^{291} in VASA (Fig. 3D) may also contribute to reorientation of the γ-phosphate. This difference may be partially a result of the interaction of Gln^{83} with the linker and through water with MAGOH (Fig. 2C). In contrast, we find it unlikely that the difference in side chain at this position could contribute significantly to the ATPase inhibition mechanism because (i) a serine in this position is also found in eIF4A, the ATPase activity of which is not inhibited by associated subunits; (ii) in eIF4AIII, the w_a water (Fig. 3) partially compensates for the Thr methyl group found in VASA; and (iii) a model with a threonine side chain in the same position in the EJC as in VASA and with the γ-phosphate in the same location as observed in the EJC does not result in unfavorable van der Waals interactions, which could induce movement of the γ-phosphate.

To further investigate how MAGOH contributes to the inhibition of the eIF4AIII ATPase, we mutated residues in the region Lys^{41} to Asp^{43} of MAGOH, which forms contacts with both domains in eIF4AIII, and deleted or mutated MAGOH residues Pro^{145} to Ile^{146}, which contact the Q motif and motif VI (Fig. 2, C and D). These mutants fall in two groups. Those that prevent EJC core assembly completely abolish ATPase inhibition by MAGOH as expected. However, some of our mutants from both regions still support EJC assembly but partially release the eIF4AIII ATPase inhibition (fig. S6), demonstrating that residues in both MAGOH regions, and perhaps in others as well, are important for ATPase inhibition.

The participation of the EJC in multiple mRNA metabolic events is made possible by its core complex constituting a dynamic binding platform for diverse processing factors. This is exemplified by the role played by the EJC in the surveillance pathway of nonsense-mediated mRNA decay (NMD). By recruiting the NMD factors Upf3b and Upf2 to newly synthesized mRNAs, the EJC serves to distinguish premature from normal translation termination codons (*21*, *22*). Because eIF4AIII patches A and G are not involved in intermolecular contacts within the EJC core (Fig. 1C), these patches may, alone or in combination with other conserved surface areas on eIF4AIII or other core EJC subunits (Fig. 4), be part of potential binding sites for peripheral EJC subunits such as the export factors REF and TAP/p15 and the NMD factor Upf3b (*2*). Given that Upf3b has been proposed to associate with the EJC through its conserved basic C-terminal domain (*23*), the acidic patch A on the surface of eIF4AIII may interact with the C-terminal domain of Upf3b.

The similarity between eIF4AIII and VASA is notable at all levels. Globally, 336 Cα atoms from both domains of eIF4AIII can be superimposed to their equivalents in VASA with a root mean square deviation of only 1.1 Å. This implies that the closed conformation of all DEAD-box proteins could be similar, although the closed conformation of other subgroups of these ATPases may be more variable. At the level of RNA binding, the almost identical recognition made by VASA and eIF4AIII is remarkable and is likely to be universal for all DEAD-box ATPases. In addition, the presence of MLN51 shows how the fundamental RNA recognition provided by the ATPase can be supplemented and regulated by additional domains in the helicases or associated proteins.

DEAD-box proteins cycle between an ATP state with high affinity for RNA and an ADP/apo state with low affinity (*4*). In both the VASA and the EJC structures, hydrogen bonds and salt bridges to RNA are formed by both domains of the ATPase, explaining the high RNA affinity of the closed conformation. In the open conformation of the adenosine diphosphate (ADP)–bound state, or the apo state, the cooperative binding between the two domains will be absent in agreement with the low RNA affinity.

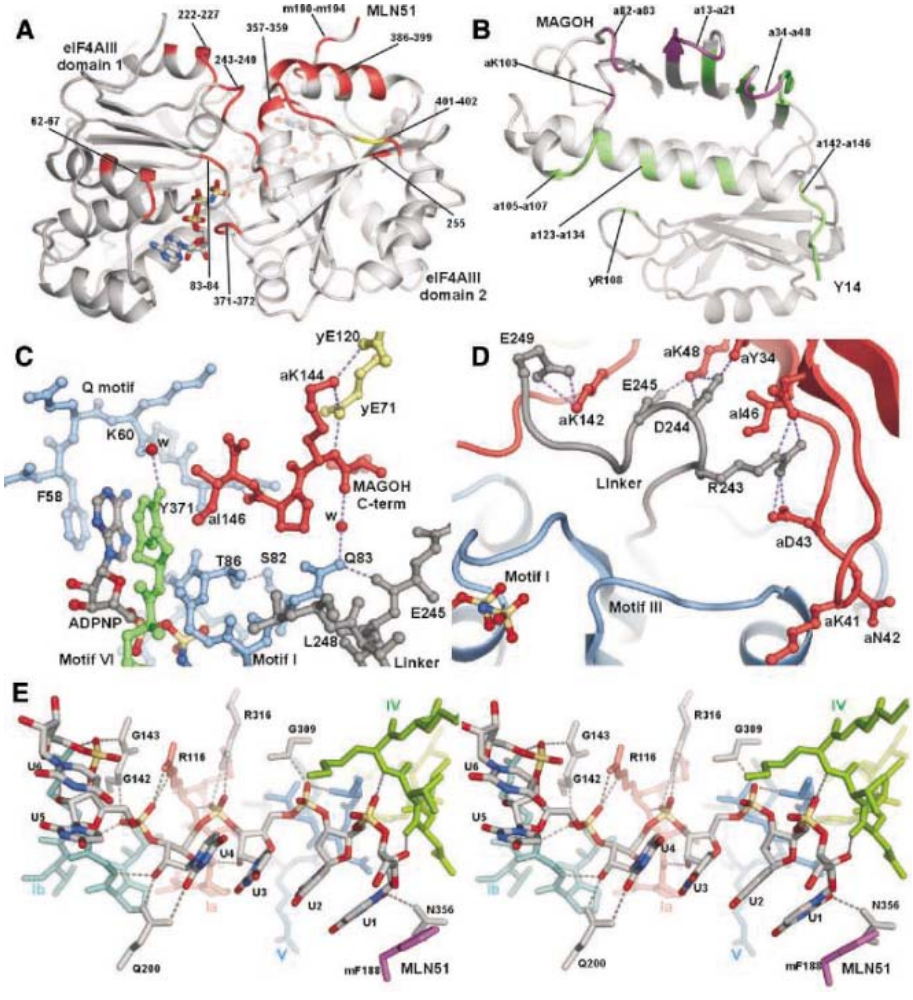

Fig. 2. Intersubunit and RNA contacts within the EJC. (**A**) Interaction footprint of MAGOH (red) and Y14 (yellow) on eIF4AIII and MLN51. (**B**) Interaction footprint of eIF4AIII (green) and MLN51 (purple) on MAGOH-Y14. (B) is rotated 180° relative to (A) around a vertical axis located between MAGOH-Y14 and eIF4AIII-MLN51. Residue numbers in MLN51, Y14, or MAGOH are preceded by m, y, or a, respectively. (**C**) Interaction of the C-terminal (C-term) residues of MAGOH with conserved motifs at the ATP binding site. Water molecules are marked w. (**D**) Packing of the linker for eIF4AIII domains 1 and 2 between MAGOH and motifs I and III at the ATP site. (**E**) Stereoview of the RNA bound to eIF4AIII with MLN51 forming the 5′ boundary of the binding pocket. Motifs Ia, Ib, IV, and V in eIF4AIII contribute to the RNA binding pocket. Residues shown in gray also participate in RNA binding but are not part of the DEAD-box motifs. Amino acid residues are labeled (*28*).

Translation is required to displace the EJC from the mRNA (*24*, *25*), and this probably requires ATP hydrolysis by eIF4AIII. Hence, the function of the eIF4AIII ATPase activity appears to be remodeling of the EJC mRNP particle, given that the structure and biochemical data clearly shows that ATP hydrolysis will induce dissociation of the EJC from RNA. The mechanism by which association between MAGOH-Y14 and eIF4AIII-MLN51 (*3*) causes ATPase inhibition can now be rationalized by our structure. Globally, MAGOH-Y14 stabilizes the closed conformation of eIF4AIII by embracing the ATPase and interacting with MLN51. A similar function has been suggested for eIF4G with respect to eIF4A (*26*), which must bind RNA and ATP in a manner almost identical to eIF4AIII. In addition, through interaction with the Q motif and the eIF4AIII domain linker, which both interact with motif I (Figs. 2C and 3A), MAGOH apparently stabilizes these regions in conformations freezing motif I and thereby the γ-phosphate. A function of the Q motif for regulating ATP binding and hydrolysis is consistent with mutational studies of the DEAD-box helicase DED1 (*27*).

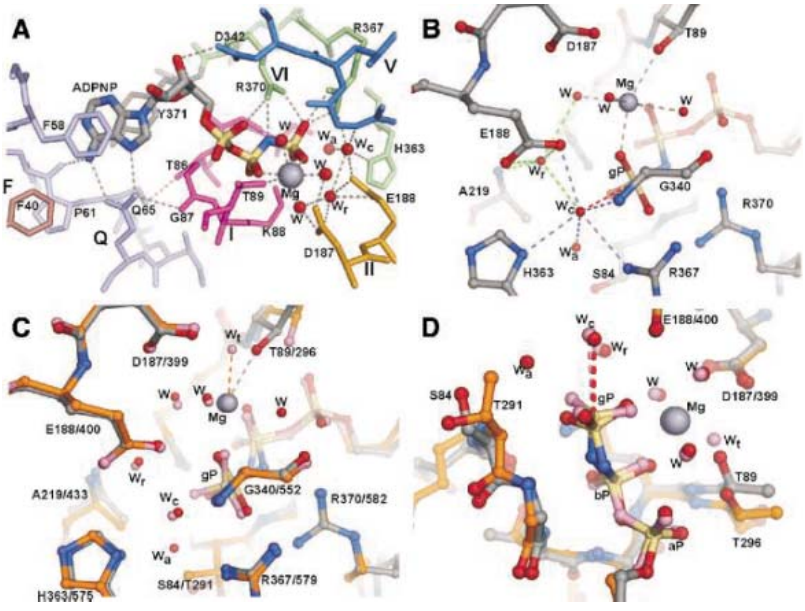

Fig. 3. The ATP hydrolysis site in DEAD-box proteins. (**A**) The eIF4AIII nucleotide binding site with residues involved in nucleotide binding colored according to the conserved motifs. Tyr^{371} is shown in gray. Water molecules are shown as red spheres. w_c, the catalytic water; w_r, a water likely to function in a proton relay; w_a, a water not found in VASA; *w*, water molecules coordinating Mg^{2+}. (**B**) Close-up of the surroundings of the γ-phosphate in the EJC. (**C**) Overlay of the EJC and VASA (*9*) structures. The w_t water is not present in the EJC but occupies the coordination position taken by eIF4AIII Thr^{89} in the EJC. Carbon atoms in VASA are colored orange; oxygen atoms, including water molecules, are pink. Residues are labeled with the eIF4AIII number followed by the VASA number. The phosphates are labeled gP, bP, and aP. (**D**) Close-up of motif I (also called P-loop or Walker A motif). The reorientation of the γ-phosphate in VASA compared with the EJC causes the geometry for the inline attack of the catalytic water to become more favorable in VASA.

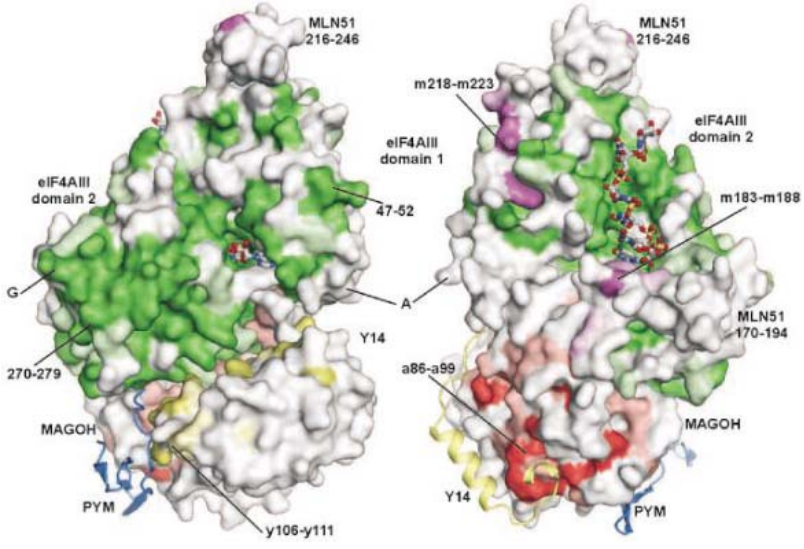

Fig. 4. Conserved surface patches of the core EJC. Conserved areas in eIF4AIII are colored green, in MLN51 purple, in Y14 yellow, and in MAGOH red. The N-terminal helix (residues 3 to 35) of Y14 from RCSB entry 1HL6 (*13*) has been docked on our structure and partially covers a highly conserved red area visible in the right panel. The ordered fragment of Pym (*28*) fits nicely into a cleft between the C-terminal helix of eIF4AIII and Y14 without overlapping with eIF4AIII and MLN51. Whether Pym contributes (*29*) or competes (*15*) for the association with the EJC core of other EJC subunits like the NMD factor Upf3b remains to be elucidated. The orientations are the same as in Fig. 1A.

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28. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Supporting Online Material
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Materials and Methods
Figs. S1 to S6
Tables S1 and S2
References

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Reversal of the TCR Stop Signal by CTLA-4

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The coreceptor cytotoxic T lymphocyte–associated antigen 4 (CTLA-4) is pivotal in regulating the threshold of signals during T cell activation, although the underlying mechanism is still not fully understood. Using in vitro migration assays and in vivo two-photon laser scanning microscopy, we showed that CTLA-4 increases T cell motility and overrides the T cell receptor (TCR)–induced stop signal required for stable conjugate formation between T cells and antigen-presenting cells. This event led to reduced contact periods between T cells and antigen-presenting cells that in turn decreased cytokine production and proliferation. These results suggest a fundamentally different model of reverse stop signaling, by which CTLA-4 modulates the threshold for T cell activation and protects against autoimmunity.

The T cell coreceptor CTLA-4 has a powerful modulatory effect on the signals that induce T cell cytokine production and proliferation (*1*, *2*). Consequently, CTLA-4–deficient (CTLA-4$^{-/-}$) mice show a profound postthymic autoimmune phenotype marked by massive tissue infiltration and organ destruction (*3*, *4*). Proposed mechanisms for the negative influence of CTLA-4 include ectodomain competition for binding of the related costimulatory molecule CD28 to CD80/86 (*5*), disruption of CD28 localization at the immunological synapse (IS) (*6*), modulation of TCR signaling by protein phosphatase 2A and the tyrosine phosphatase SHP-2 (*7*–*9*), and interference with the expression or composition of lipid rafts on the surface of T cells (*10*–*12*). In addition, CTLA-4 engagement of CD80/86 on dendritic cells (DCs) can induce the release of indoleamine 2,3-dioxygenase (*13*), whereas CD4$^+$CD25$^+$ regulatory T cells can modulate disease in the CTLA-4$^{-/-}$ mouse (*14*–*16*). Antibodies to CTLA-4 (anti–CTLA-4) and CTLA-4–immunoglobulin have been used as therapeutics in the modulation of autoimmunity, transplantation, and tumor immunotherapy (*17*, *18*).

CTLA-4 is a potent direct activator and a TCR coactivator of LFA-1 integrin clustering and adhesion (*19*). To assess whether CTLA-4 could also influence integrin-dependent motility, we tracked preactivated CTLA-4$^{+/+}$ and CTLA-4$^{-/-}$ T cells for movement on plates coated with the LFA-1 ligand intercellular adhesion molecule–1 (ICAM-1) (Fig. 1). In contrast to untreated CTLA-4$^{+/+}$ and CTLA-4$^{-/-}$ cells, which migrated at similar speeds (Fig. 1, A, C, and E), CTLA-4$^{+/+}$ cells treated with anti–CTLA-4 increased motility (Fig. 1, B versus A). As anticipated, the effects of anti–CTLA-4 were not observed with CTLA-4$^{-/-}$ T cells (Fig. 1, D versus C; E). Thus, in addition to increasing LFA-1 adhesion (*19*), CTLA-4 readily increased the movement of T cells.

TCR ligation reduces or arrests T cell motility (i.e., the stop signal), an event that is required for stable T cell conjugate formation with antigen-presenting cells (APCs) and efficient activation (*20*–*22*). Given that CTLA-4 increased T cell motility, we assessed whether the coreceptor could override this stop signal. Preactivated murine and human T cells were monitored for 20 min after exposure to anti-

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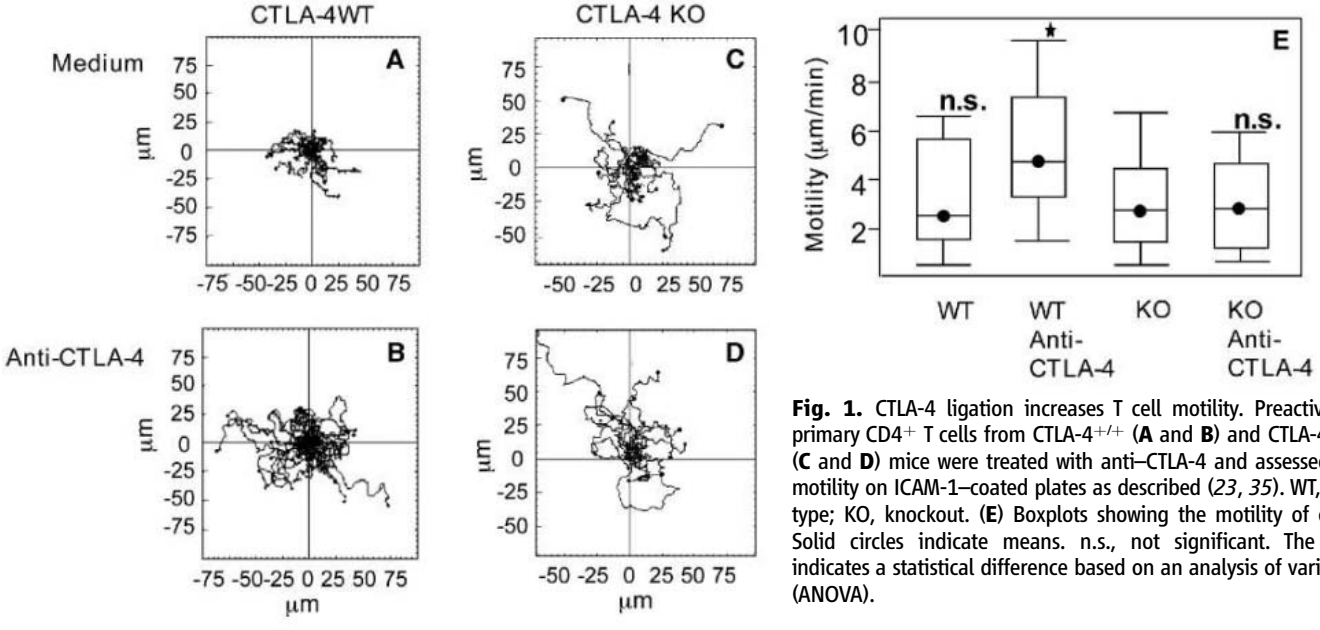

Fig. 1. CTLA-4 ligation increases T cell motility. Preactivated primary CD4$^+$ T cells from CTLA-4$^{+/+}$ (**A** and **B**) and CTLA-4$^{-/-}$ (**C** and **D**) mice were treated with anti–CTLA-4 and assessed for motility on ICAM-1–coated plates as described (*23*, *35*). WT, wild type; KO, knockout. (**E**) Boxplots showing the motility of cells. Solid circles indicate means. n.s., not significant. The star indicates a statistical difference based on an analysis of variance (ANOVA).

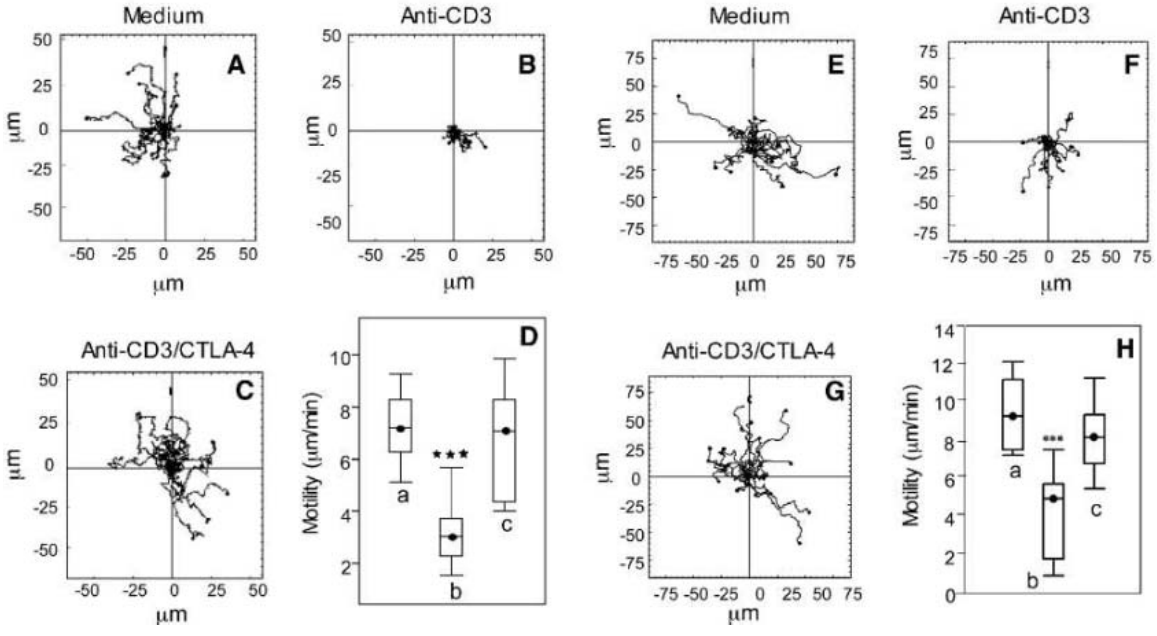

Fig. 2. (**A** to **D**) CTLA-4 ligation reverses the TCR-induced stop signal of mouse primary T cells. Preactivated primary CD4$^+$ T cells were stimulated with anti-CD3 or anti–CD3/CTLA-4 and assessed for motility on ICAM-1–coated plates as described (*23*, *35*). (A) media control; (B) anti-CD3; (C) anti-CD3/CTLA-4; (D) boxplots of motility patterns. Solid circles indicate means. a, medium; b, anti-CD3; c, anti–CD3/CTLA-4. The three stars represent a statistical difference based on ANOVA. (**E** to **H**) CTLA-4 ligation reverses the TCR-induced stop signal of human primary T cells. Preactivated human peripheral T cells were stimulated with anti-CD3 or anti–CD3/CTLA-4 and were monitored for movement as described (*23*). (E) media control; (F) anti-CD3; (G) anti–CD3/CTLA-4; (H) boxplots of motility patterns. Solid circles indicate means. a, medium; b, anti-CD3; c, anti–CD3/CTLA-4. The three asterisks indicate a statistical difference based on ANOVA.

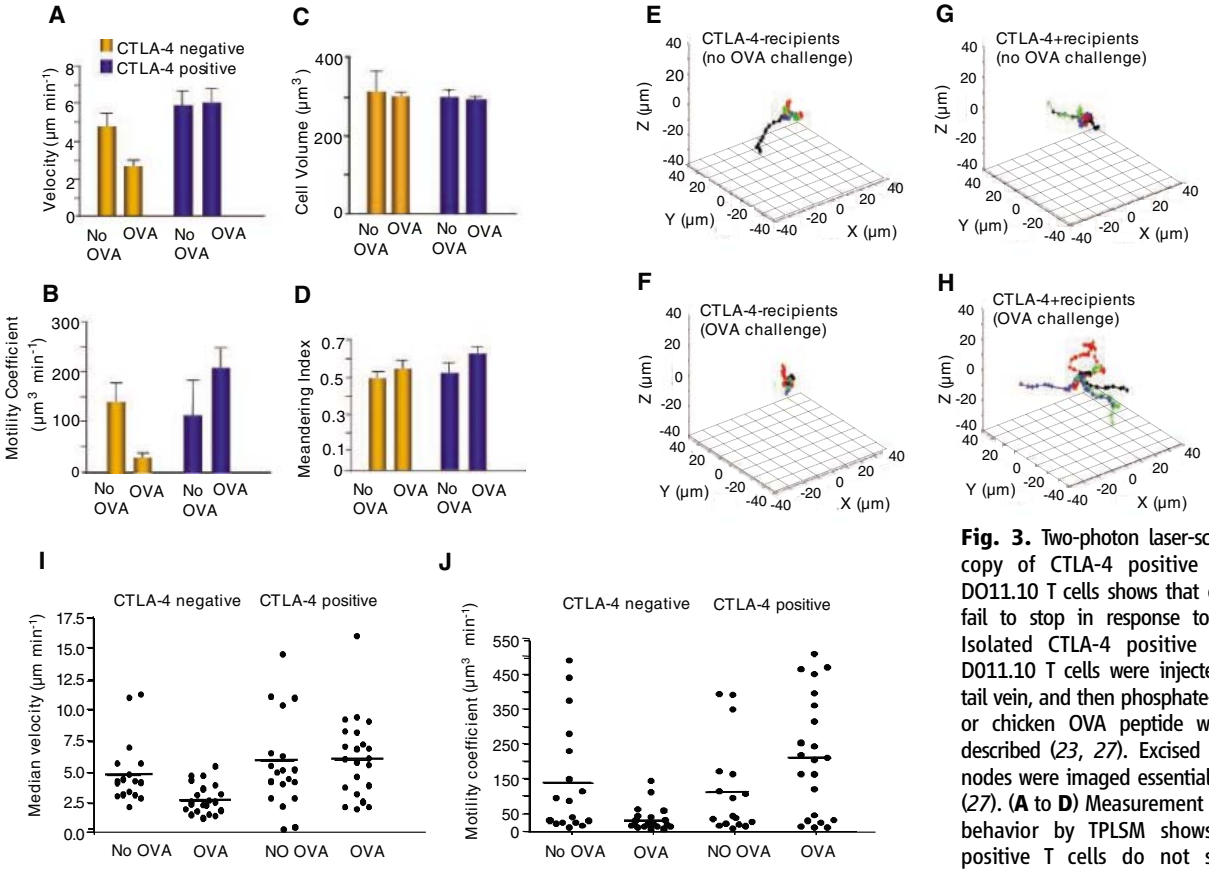

Fig. 3. Two-photon laser-scanning microscopy of CTLA-4 positive and negative DO11.10 T cells shows that expressing cells fail to stop in response to OVA peptide. Isolated CTLA-4 positive and negative D011.10 T cells were injected through the tail vein, and then phosphate-buffered saline or chicken OVA peptide was injected as described (*23*, *27*). Excised cervical lymph nodes were imaged essentially as described (*27*). (**A** to **D**) Measurement of in vivo T cell behavior by TPLSM shows that CTLA-4 positive T cells do not slow down in response to peptide. Error bars represent SEM. Data in movies S1 to S4 were analyzed with PicViewer and Volocity software. (A) velocity; (B) motility coefficient; (C) volumes; (D) meandering factor. (**E** to **H**) Representative movement of CTLA-4 positive and negative cells observed by TPLSM. The different color traces represent single cells. The behavior of T cells in the presence and absence of peptide antigen was monitored with Volocity software. (E) CTLA-4$^-$ recipients with no OVA; (F) CTLA-4$^-$ recipients with OVA; (G) CTLA-4$^+$ recipients with no OVA; (H) CTLA-4$^+$ recipients with OVA. (**I** and **J**) Dot blot analysis of individual CTLA-4 positive and negative cells in response to peptide. The tracing of individual T cells showed heterogeneity with a reduction in the median velocity (μm min^{-1}) and motility coefficient (μm^3 min^{-1}) of CTLA-4 negative cells in response to injected peptide, whereas CTLA-4 positive cells failed to reduce motility. Horizontal bars indicate SEM.

CD3 in the presence or absence of anti–CTLA-4. In contrast to the ligation of anti-CD3, which reduced the movement of mouse and human primary T cells (Fig. 2, B versus A and F versus E), the coligation of CTLA-4 and anti-CD3 reversed the arrest such that treated and untreated cells moved at similar speeds (Fig. 2, C versus A and G versus E; D and H). Similar effects were seen with the CTLA-4–transfected T cell hybridoma DC27.10 (fig. S1). These findings showed that CTLA-4 can override the stop signal induced by the antigen receptor complex.

To extend our findings to an in vivo setting, we separated preactivated CTLA-4 positive and negative $CD4^+$ T cells transgenic for the DO11.10 TCR using beads coated with anti–CTLA-4 (*23*). These cells were then labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) and tracked in cervical lymph nodes by two-photon laser scanning microscopy (TPLSM) (Fig. 3). T cells in mice injected with antigen have been shown to undergo changes in motility involving initial transient serial encounters, followed by a phase of slowing and stable contacts and then by a return to high motility (*22*, *24*–*27*). In our study, CTLA-4 positive and negative T cells were monitored at 15, 20, and 24 hours after injection with ovalbumin (OVA) peptide. The first noticeable difference between cells was a more extended, polarized appearance of CTLA-4 positive cells (fig. S2 and movies S1 to S4). In the absence of peptide, CTLA-4–expressing and –nonexpressing T cells showed a random walk pattern with similar motilities (Fig. 3, A and E versus G). After the OVA peptide injection, CTLA-4 negative cells showed the anticipated decrease in mean motility (Fig. 3, A and F versus E). By contrast, the motility of CTLA-4 positive cells remained unchanged and occasionally increased in the presence of OVA peptide (Fig. 3, A and H versus F; fig. S2, movies S1 to S4). The three-dimensional motility coefficient showed a reduction from 140 to 29.7 $\mu m^3\ min^{-1}$ for CTLA-4 negative cells and an increase from 114 to 210 $\mu m^3\ min^{-1}$ for CTLA-4 positive cells (Fig. 3B). Dot plot analysis showed some heterogeneity in the motility of individual cells, although the mean motility of CTLA-4 positive cells remained unchanged in the presence of peptide (Fig. 3, I and J). Despite this result, the two subsets had similar volumes (Fig. 3C) and meandering indices (Fig. 3D). This resistance of CTLA-4 positive cells to antigen-induced slowing was observed at all times examined. These experiments revealed that CTLA-4 positive cells fail to stop or slow down in response to the in vivo peptide challenge.

Because the slowing of motility is required for stable T cell–APC conjugate formation (*20*–*22*, *26*, *27*), the reversal of the stop signal by CTLA-4 suggested that the coreceptor might reduce the period of interaction. To test this conjecture, we incubated CTLA-4 positive and negative DO11.10 T cells with a CFSE-labeled B cell line, A20 (Fig. 4A), or labeled mature DCs (Fig. 4B). APCs were left untreated or were preincubated with OVA peptide. Individual T cells were then monitored for the duration of APC binding over a 1200-s period. Whereas CTLA-4 negative T cells incubated with APCs without peptide showed short-term interactions (75% of which were less than 400 s), the addition of OVA peptide induced longer term interactions (60 to 70% were more than 600 s). In contrast, CTLA-4 positive T cells failed to form long-term interactions even in the presence of antigen. Instead, like those cells unexposed to peptide, CTLA-4 positive cells undertook short-term interactions. We observed this effect when A20 B cells or DCs were used as APCs (Fig. 4, A and B). In turn, these shorter dwell periods (Fig. 4C) resulted in lower interleukin-2 (IL-2) production (Fig. 4D) and proliferation (Fig. 4E). CTLA-4 expression therefore led to more transient interactions with APCs and a reduction in T cell activation.

Overall, our observations support a reverse stop-signaling mechanism for modulating the threshold of T cell activation by CTLA-4, which is distinct from previous models of CTLA-4 function. By limiting T cell–APC contact times, CTLA-4 would reduce the efficiency of the major histocompatibility complex (MHC)–peptide presentation and the number of TCR ligation events resulting in reduced signaling and activation. Shorter dwell times could also alter receptor rearrangements at the IS and account for the reduced localization of CD28 (*6*). In this way, CTLA-4 may be tailored to regulate the secondary responses of effectors such as cytotoxic T lymphocytes, whose shorter dwell times are sufficient to elicit cytotoxicity. More transient interactions may in turn increase the frequency of contacts between effector and target cells. CTLA-4 positive cells may also compete with CTLA-4 negative cells to inhibit longer term conjugation with APCs and facilitate a rapid exit from lymph nodes and reentry to the circulation for migration to sites of inflammation.

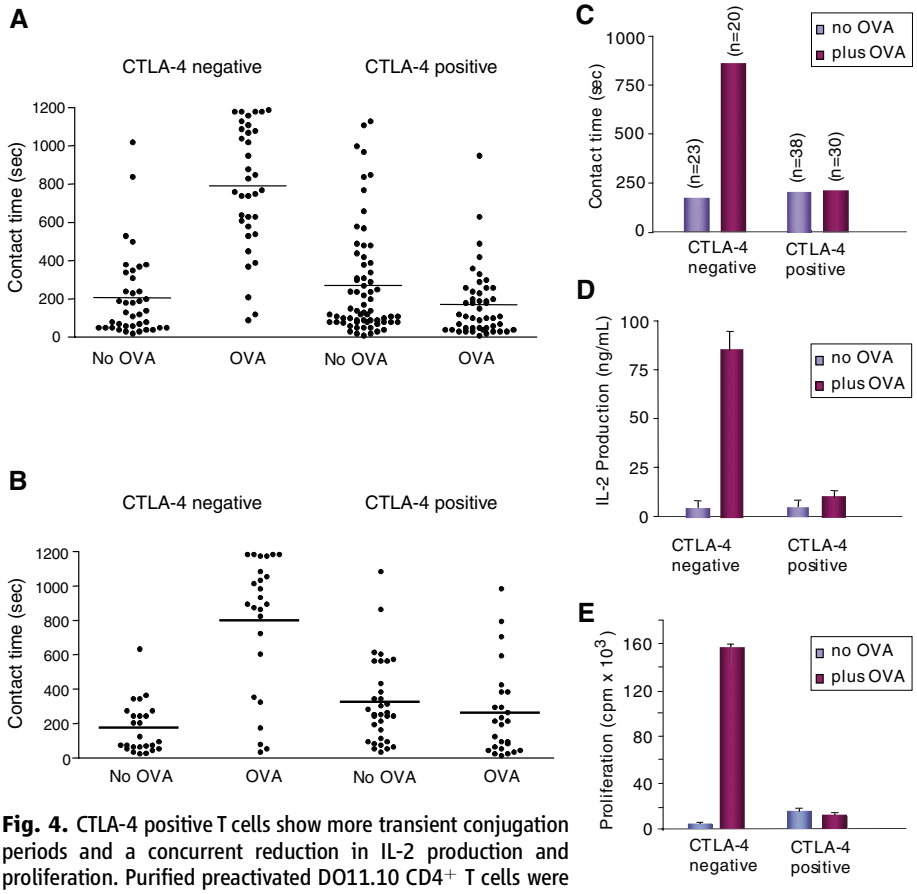

Fig. 4. CTLA-4 positive T cells show more transient conjugation periods and a concurrent reduction in IL-2 production and proliferation. Purified preactivated DO11.10 $CD4^+$ T cells were then incubated with A20 B cells or mature DCs preloaded with OVA peptide as described (*35*). (**A** and **B**) Comparison of the conjugation times of CTLA-4 positive and negative cells in the presence and absence of OVA peptide. Whereas the conjugation time of CTLA-4 negative cells was extended in response to added peptide, the conjugation time of CTLA-4 positive cells was unaffected. A20 B cells and mature DCs were used as APCs in (A) and (B), respectively. Horizontal bars indicate SEM. (**C** to **E**) Reduced conjugation periods of CTLA-4 positive cells correlate with reduced levels of IL-2 production and proliferation. IL-2 production and proliferation were measured as described (*19*). Representative experiments showing differences in the period of conjugation (C), level of IL-2 production (D), and proliferation (E) for CTLA-4 negative and positive cells. Error bars in (D) and (E) represent SEM. cpm, counts per minute.

The role of CTLA-4 as a gatekeeper of conjugation may also account for the connection with autoimmunity (*3*, *4*). Reduced conjugation might protect against prolonged contact periods that allow for responses to lower-affinity autoantigen. Increased LFA-1–mediated adhesion in the absence of increased motility allows for responses to low-affinity subthreshold agonist including self-MHC molecules (*28*–*30*). This may also explain the reported requirement for anergy induction of the T cell (*31*, *32*). In certain settings, by limiting dwell times, suboptimal or altered signaling may result in nonresponsiveness. Indeed, tolerance induction is accompanied by less stable DC–T cell interactions (*26*, *33*), smaller clusters (*27*), and a rapid restoration of motility (*33*). Regulatory T cells can also limit T cell–APC contact times (*34*). Further studies will more precisely elucidate the role of altered motility and conjugation in the full range of functions regulated by the coreceptor.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1131078/DC1
Materials and Methods
Figs. S1 to S3
Movies S1 to S4

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Dok-7 Mutations Underlie a Neuromuscular Junction Synaptopathy

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Congenital myasthenic syndromes (CMSs) are a group of inherited disorders of neuromuscular transmission characterized by fatigable muscle weakness. One major subgroup of patients shows a characteristic "limb girdle" pattern of muscle weakness, in which the muscles have small, simplified neuromuscular junctions but normal acetylcholine receptor and acetylcholinesterase function. We showed that recessive inheritance of mutations in Dok-7, which result in a defective structure of the neuromuscular junction, is a cause of CMS with proximal muscle weakness.

In congenital myasthenic syndromes (CMSs), the safety margin for neuromuscular transmission is compromised, resulting in characteristic myasthenic fatigable muscle weakness. A search of candidate genes has resulted in the identification of mutations in nine genes that encode proteins at the neuromuscular junction (NMJ) (*1*), but there are additional CMSs for which the underlying molecular defect remains to be defined (*2*). One of these syndromes causes a characteristic "limb girdle" pattern of muscle weakness. Because muscle biopsies have not found clear-cut defects in the functional components of signal transmission but revealed abnormally small NMJs (*3*), it was concluded that this disorder may arise from the defective formation or maintenance of the synaptic structure of the NMJ.

Studies, mainly with knock-out mice, have elucidated key processes in the initiation and maintenance of the specialized postsynaptic structures at the NMJ (*4*). Agrin, which is released from the nerve terminal, activates muscle-specific tyrosine kinase (MuSK) located in the postsynaptic membrane, which leads to the precise aggregation and localization of the acetylcholine receptors (AChRs) through their association with the cytoplasmic anchoring protein rapsyn. However, additional key components contribute to this pathway (*5*–*7*). Dok-7, a member of the Dok family of cytoplasmic molecules, can induce the aneural activation of MuSK and the subsequent clustering of AChR in cultured myotubes (*8*).

We screened genomic DNA from patients with suspected CMS for mutations within the seven coding exons of the human *dok-7* gene by amplification using polymerase chain reaction (PCR) and bidirectional DNA sequencing [see supporting online material (SOM)], and we identified frameshift mutations in 16 unrelated patients. In three additional patients, a frameshift mutation was identified in combination with a nonsense mutation, a splice site mutation, and a missense change of a conserved residue, $Gly^{180} \rightarrow Ala^{180}$ (G180A), within the phosphotyrosine binding domain (PTB) (Fig. 1A and Table 1). In two other patients, we identified a frameshift mutation but have yet to identify a second mutation. Sixteen of the 21 patients that had frameshift mutations harbored 1124_1127dupTGCC, indicating that this was a common mutation in this group of patients and that allele-specific PCR can facilitate genetic screening. When DNA from family members was available, we observed that the disease cosegregated with recessive inheritance of the Dok-7 frameshift mutations (Fig. 1B). Sequencing of the entire coding region of Dok-7 from 25 control individuals revealed no frameshift mutations, and allele-specific PCR did not detect 1124_1127dupTGCC in 204 control chromosomes. Similarly, BsaH I restriction endonuclease digests of amplicons of exon 5 showed the segregation of G180A with recessive disease inheritance and did not detect G180A in 200 control chromosomes, suggesting that this mutation is

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also likely to be pathogenic. When DNA from family members was not available, subcloning and sequencing of amplicons from genomic DNA, or cDNA derived from muscle biopsies, demonstrated that the mutations were on separate alleles. Previous screening of DNA from index cases 2 to 5, 8, 11, 20, and 21 did not detect mutations in the genes encoding the AChR subunits (*CHRNA*, *CHRNB*, *CHRND*, and *CHRNE*), *RAPSN*, *COLQ*, or *CHAT*, where defects are known to cause CMS. Thus, we identified at least 19 unrelated CMS patients in which disease was associated with recessive mutations in Dok-7.

Because mice lacking Dok-7 die shortly after birth (*8*), we determined how the allelic C-terminal truncations that were seen in the CMS patients affect Dok-7 function. Dok-7 harboring the common mutation 1124_1127dupTGCC was cloned into pcDNA3.1 (Fig. 2A) and coexpressed with Myc-tagged MuSK in human embryonic kidney (HEK) 293 cells. The mutant Dok-7 showed robust expression and induced phosphorylation of MuSK (fig. S1A). Pull-down assays with an antibody to Myc (anti-Myc) showed coimmunoprecipitation of MuSK and mutant Dok-7 (fig. S1B), indicating that the C-terminal truncation does not disrupt Dok-7–MuSK interaction in the HEK 293 cells. However, because the regulatory interaction of Dok-7 with MuSK is more sensitive to deletion mutations in Dok-7 in differentiated myotubes (*8*), we tested whether mutant Dok-7 affects MuSK function when overexpressed in C2C12 myotubes. Although the mutant Dok-7 again showed robust expression, immunoblots conducted after pull-down assays with anti-MuSK or α-bungarotoxin–Sepharose (which binds AChRs) showed reduced phosphorylation of MuSK (one-tailed paired *t* test, $P = 0.037$, three independent experiments) and of the muscle AChR β subunit ($P = 0.016$, three independent experiments), which is a known downstream target of MuSK, indicating partially impaired MuSK function (Fig. 2, B and C).

We next analyzed the AChR clusters that were induced in C2C12 myotubes after the expression of wild-type or mutant Dok-7–1124_1127dupTGCC. Compared to the wild type, Dok-7–1124_1127dupTGCC induced the formation of fewer clusters that were 5 μm or greater in size [90 ± 9.41 versus 37 ± 5.29 clusters per 10 fields; mean ± SD; one-way analysis of variance (ANOVA); $P < 0.0001$; wild type versus Dok-7–1124_1127dupTGCC; three independent experiments], with a shift toward smaller clusters (Fig. 2D, two-way repeated measures ANOVA, $P < 0.001$, three independent experiments). AChR clusters formed in cultured myotubes show a variety of morphologies, including plaque-shaped, perforated, c-shaped, and branched types (*9*), that may reflect the maturation of the NMJ seen in vivo. The branched shape of the clusters corresponds most closely to the morphology of the mature NMJ (*9*). Analysis of clusters induced by the Dok-7–1124_1127dupTGCC mutation, as compared to the wild type, showed a significant reduction in the number of branched-type plaques (Fig. 2E, one-tailed paired *t* test, $P = 0.026$, three independent experiments), suggesting that the mutation attenuates the maturation of the synaptic structure. Thus, in cultured myotubes, the truncation of Dok-7 by 1124_1127dupTGCC impairs MuSK activity and its ability to shape the specialization of postsynaptic structures.

Clinical examination of 19 out of 21 index cases and the affected brother of case 11 was undertaken in Oxford or Munich (table S1). All of the patients displayed electromyographic evidence of a defect in neuromuscular transmission (determined by abnormal decrement and/or abnormal single-fiber studies), and all but three patients developed weakness within the first five years of life. The clinical onset of disease was generally characterized by difficulty in walking after initially achieving normal walking milestones. Features typically seen in patients with rapsyn mutations, such as congenital joint deformity and squint, were not present. In adulthood, a proximal weakness of the affected patients' upper and lower extremities was evident, and most had weakness in the trunk and neck regions. All of the patients had weak facial muscles, and all but two patients had ptosis. Eye movements were generally unaffected. Anticholinesterase medication either had no effect or made the weakness worse, although a short-lived initial response was occasionally seen.

Table 1. Mutations in Dok-7 identified in CMS patients. c., cDNA nucleotide numbering from the adenine of the initiating ATG codon; p., protein sequence; fs, frameshift; IVS, intron; n.d., not determined; dash, mutation not yet identified. The number that comes after the "X" indicates the number of frameshift missense amino acids that are present before a nonsense codon.

Index case	Ethnic origin	Identified mutations	Alteration in coding sequence	Dok-7 exon
1	UK, white	c.1124_1127dupTGCC	p.Pro376ProfsX30	7
		c.1339_1342dupCTGG	p.Gly447AlafsX70	7
2	UK, white	c.1124_1127dupTGCC	p.Pro376ProfsX30	7
		c.1124_1127dupTGCC	p.Pro376ProfsX30	7
3	UK, white	c.1124_1127dupTGCC	p.Pro376ProfsX30	7
		c.1263insC	p.Ser422LeufsX94	7
4	UK, white	c.1124_1127dupTGCC	p.Pro376ProfsX30	7
		c.1263insC	p.Ser422LeufsX94	7
5	UK, white	c.1124_1127dupTGCC	p.Pro376ProfsX30	7
		c.1263insC	p.Ser422LeufsX94	7
6	UK, white	c.1124_1127dupTGCC	p.Pro376ProfsX30	7
		c.1124_1127dupTGCC	p.Pro376ProfsX30	7
7	Indian subcontinent	c.548_551delTCCT	p.Phe183CysfsX61	5
		c.1124_1127dupTGCC	p.Pro376ProfsX30	7
8	UK, white	c.1143insC	p.Glu382ArgfsX24	7
		c.1143insC	p.Glu382ArgfsX24	7
9	UK, white	c.1143insC	p.Glu382ArgfsX24	7
		c.1339_1342dupCTGG	p.Gly447AlafsX70	7
10	UK, white	c.1124_1127dupTGCC	p.Pro376ProfsX30	7
		c.1339_1342dupCTGG	p.Gly447AlafsX70	7
11	UK, white	c.539G>C	G180A	5
		c.1124_1127dupTGCC	p.Pro376ProfsX30	7
12	UK, white	c.1124_1127dupTGCC	p.Pro376ProfsX30	7
		c.1124_1127dupTGCC	p.Pro376ProfsX30	7
13	UK, white	c.1124_1127dupTGCC	p.Pro376ProfsX30	7
		-	-	-
14	UK, white	1143insC	p.Glu382ArgfsX24	7
		-	-	-
15	Finnish	c.1378insC	p.Glu460ProfsX58	7
		c.1508insC	p.Pro503ProfsX15	7
16	French-Canadian	c.1263insC	p.Ser422LeufsX94	7
		c.1124_1127dupTGCC	p.Pro376ProfsX30	7
17	Portuguese	c.1357_1370del14	p.Arg452ArgfsX61	7
		c.1124_1127dupTGCC	p.Pro376ProfsX30	7
18	Spanish	IVS2-1G>T	n.d.	intron 2
		c.1124_1127dupTGCC	p.Pro376ProfsX30	7
19	German	c.1124_1127dupTGCC	p.Pro376ProfsX30	7
		c.1124_1127dupTGCC	p.Pro376ProfsX30	7
20	German	c.601C>T	p.R201X	5
		c.1124_1127dupTGCC	p.Pro376ProfsX30	7
21	Spanish	c.1124_1127dupTGCC	p.Pro376ProfsX30	7
		c.1124_1127dupTGCC	p.Pro376ProfsX30	7

An analysis of motorpoint muscle biopsies from a cohort of CMS patients with limb girdle proximal weakness showed that many features of the NMJ were normal, including the quantal release per unit area of synaptic content and the size and kinetics of the miniature end-plate currents (*3*). However, two major abnormalities were identified: a reduced size of the NMJs (Tables 2 and 3) and reduced postsynaptic folding (*3*). We identified C-terminal domain frameshift mutations in Dok-7 in DNA available from six out of seven patients included in the study by Slater *et al.* (Table 2) and from three patients with reduced NMJ size that were studied in Oxford (Table 3). The postsynaptic fold index in biopsies from patients harboring Dok-7 mutations that were studied at Newcastle was 5.51 ± 1.2 (mean ± SD, n = 7) versus 8.05 ± 1.34 (mean ± SD, n = 8) for control patients.

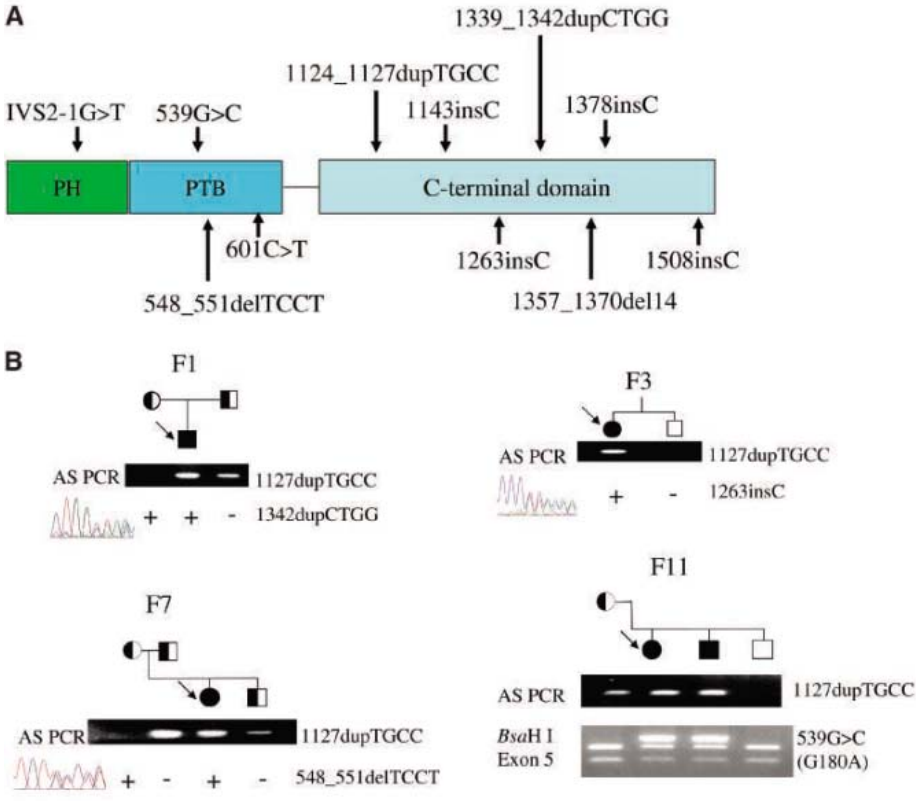

Fig. 1. Identification of mutations in Dok-7. (**A**) Position of mutations (arrows) in the functional domains of Dok-7. PH, pleckstrin homology domain. The C-terminal domain contains the target motifs of the Src homology 2 domain. (**B**) Segregation of disease with recessive inheritance of mutant alleles. Colored traces show DNA sequencing electrophoretograms of respective frameshift mutations. Mutation 1124_1127dupTGCC is labeled as 1127dupTGCC and mutation 1339_1342dupCTGG as 1342dupCTGG. AS PCR, allele-specific PCR for 1127dupTGCC. The presence (plus sign) or absence (minus sign) of a frameshift mutation was detected by bidirectional DNA sequencing as shown in the electrophoretograms; BsaH I restriction digests were used to detect 539G>C in exon 5. Affected family members (black symbols) carry two mutant alleles. Unaffected members harbor one (half-black symbols) or no (white symbols) mutant alleles. F1, F3, F7, and F11 refer to the respective families of patients in Table 1. Arrows indicate the index cases.

Our results demonstrate that mutations in Dok-7 affect the size and structure of the NMJ and underlie a CMS with a characteristic pattern of weakness, particularly affecting the proximal muscle groups. Impaired activation of MuSK is implicated as the pathogenic mechanism in these patients with Dok-7 mutations. The reduction in NMJ size, and hence efficacy, that results from mutations affecting the C-terminal region of Dok-7 could not have been predicted from earlier experiments, in which the complete inactivation of the gene encoding Dok-7 in mice virtually abolished NMJ formation (*8*). Many NMJ-associated proteins, including rapsyn (*10*), ColQ (*11*), and various different kinases such as Abl and Src/Fyn (*12*, *13*), have been proposed as interacting partners with MuSK. The truncation of the C-terminal end of Dok-7 may reduce MuSK catalytic activity directly, affect the docking of other signal-transducing molecules, and/or affect the binding of MuSK to other NMJ-associated proteins. Subsequently, the pre- and postsynaptic structures are affected, leading to the reduced synaptic size.

Because the onset of symptoms resulting from Dok-7 mutations is usually observed in early childhood and not at birth, the mutations probably have little pathogenic effect on initial synapse formation but instead exert their effects through aberrant synaptic maturation or maintenance. This suggestion is consistent with our functional studies showing that 1124_1127dupTGCC impairs the maturation of the postsynaptic structure in cultured myotubes. Dok-7 that harbored a truncated C-terminal region induced MuSK activation during the early stages of the differentiation of C2C12 cells into myotubes but not when the myotubes were fully differentiated (*8*).

Mutations in Dok-7 appear to be a common cause of CMS in patients of different European or Indian ethnic origins (Table 1). Genetic screening of exon 7 in *dok-7* should facilitate the diagnosis of this disorder, although, because a limb girdle pattern of muscle weakness has been reported for other myasthenic disorders (*14*–*16*),

Table 2. Vastus lateralis biopsies from patients harboring mutations in Dok-7 studied in Newcastle. For the index cases, the numbers refer to those in Table 1, in which the index case mutations are identified. The number associated with LGM (limb-girdle myasthenia) refers to patients whose vastus lateralis biopsies were reported in (*3*); patient 1 was not included in that study. The total area of acetylcholinesterase (AChE)–stained NMJs was measured. ns, not significant; MEPP, miniature end-plate potential; MEPC, miniature end-plate current.

Index case	Sex, age at biopsy	AChRs/NMJ ($\times 10^7$)	Total AChE area (μm^2)	AChRs/AChE area	MEPP amplitude (mV)	MEPC amplitude (nA)	Quantal content (m)
1	M, 22	1.06	69.6	0.015	0.64	nd	13.8
8 (LGM1)	F, 12	nd	109.0	nd	0.45	2.04	14.7
9 (LGM3)	M, 14	nd	82.1	nd	0.43	3.84	12.4
11 (LGM8)	F, 38	0.65	47.1	0.014	0.62	6.13	13.2
12 (LGM5)	M, 22	1.26	107.9	0.012	0.56	5.40	4.8
13 (LGM4)	F, 26	0.90	78.6	0.012	0.51	4.41	11.5
14 (LGM7)	M, 20	0.46	82.4	0.006	0.33	4.26	15.5
Mean ± SD (n)		0.87 ± 0.32 (5)	82.3 ± 21.5 (7)	0.012 ± 0.003 (5)	0.51 ± 0.11 (7)	4.35 ± 1.41 (6)	12.3 ± 3.6 (7)
Controls, mean ± SD (n)		2.34 ± 0.4 (2)	146.8 ± 26.9 (8)	0.016 ± 0.006 (2)	0.71 ± 0.19 (8)	3.59 ± 0.47 (6)	21.2 ± 4.8 (8)
P, unpaired t test		0.003	0.009	0.23, ns	0.023	0.24, ns	0.0013

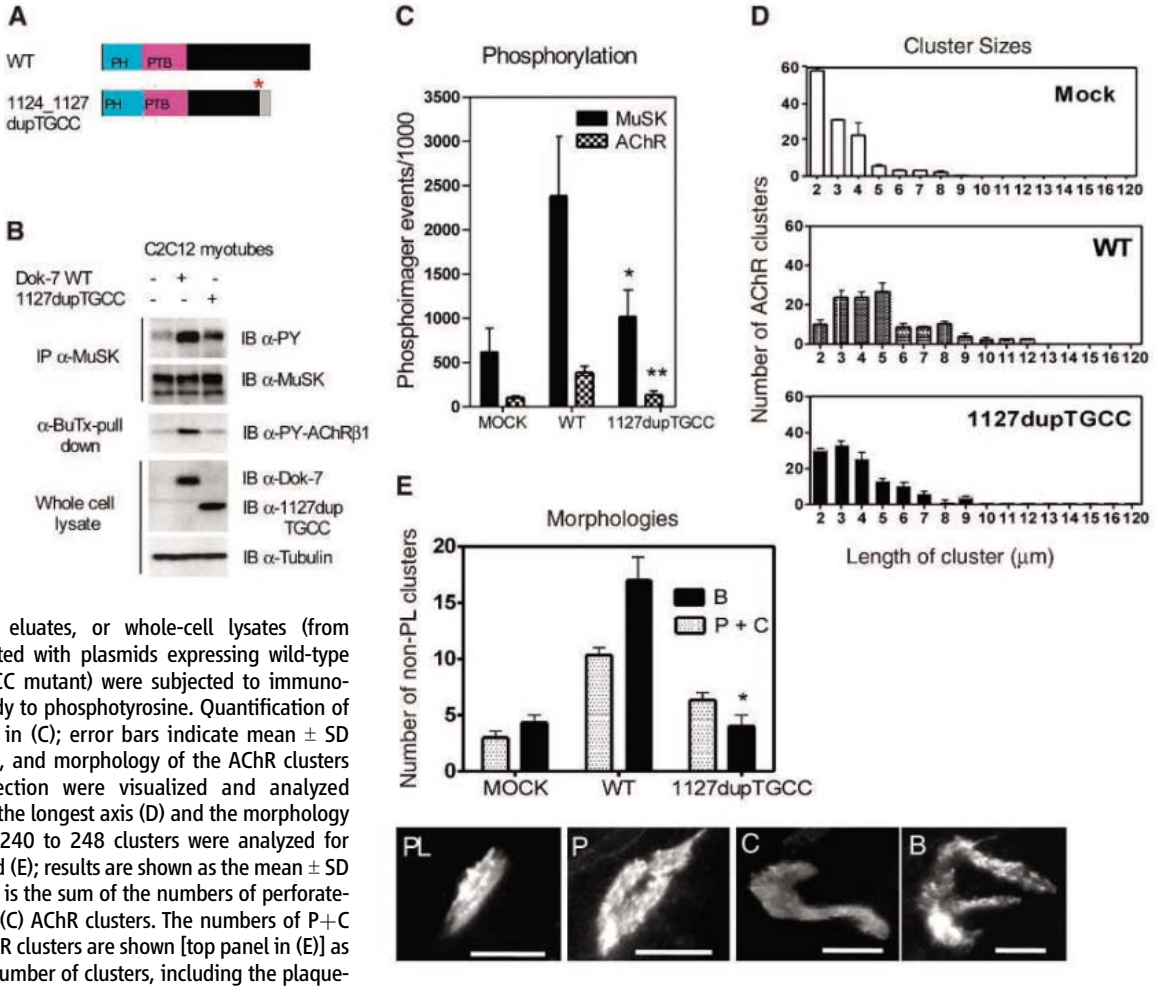

Fig. 2. The 1124_1127dupTGCC mutation (1127dupTGCC) impairs Dok-7–induced postsynaptic specialization in myotubes. (**A**) Schematic representation of wild-type Dok-7 (WT) and its mutant harboring a 1124_1127dupTGCC mutation, showing the position of the frameshift (indicated by the asterisk) and the resulting region of missense amino acids (light gray area). (**B** to **E**) The 1127dupTGCC mutation impairs Dok-7–induced MuSK activation and the subsequent AChR clustering in myotubes. (B) Anti-MuSK immunoprecipitates (IP), α-bungarotoxin (BuTx) eluates, or whole-cell lysates (from C2C12 myotubes transfected with plasmids expressing wild-type Dok-7 or the 1127dupTGCC mutant) were subjected to immunoblotting (IB). α-PY, antibody to phosphotyrosine. Quantification of phosphorylation is shown in (C); error bars indicate mean ± SD (n = 3). The number, size, and morphology of the AChR clusters induced by each transfection were visualized and analyzed according to the length of the longest axis (D) and the morphology of individual clusters (E). 240 to 248 clusters were analyzed for each transfection in (D) and (E); results are shown as the mean ± SD of three experiments. P+C is the sum of the numbers of perforate-type (P) and c-shape type (C) AChR clusters. The numbers of P+C and branched-type (B) AChR clusters are shown [top panel in (E)] as a percentage of the total number of clusters, including the plaque-type (PL) cluster. Representative clusters are shown in the bottom panels of (E). Scale bars, 20 μm.

Table 3. Intercostal muscle biopsies in patients harboring mutations in Dok-7 studied in Oxford. Index case numbers refer to those in Table 1, in which the index case mutations are identified. The length of AChE-stained NMJs was measured as length in sarcomeres, where one sarcomere equals 3.2 μm.

Index case	Sex, age at biopsy	AChRs/NMJ ($\times 10^7$)	Length of AChE stained area (μm)	AChRs/AChE length	MEPP amplitude (mV)	MEPC amplitude (nA)	Quantal content (m)
2*	M, 41	0.84	17.6	0.048	0.32†	nd	nd
3	F, 53	0.90	15.3	0.059	0.50	nd	14
4*	F, 44	0.60	22.7	0.027	0.56	nd	nd
Controls, mean ± SD (n)		1.2 ± 0.3 (7)	24 ± 2.9 (3)	0.042 (3)	0.8 ± 0.1 (4)		20 ± 3.2 (4)

*Data reported in (*18*). †Determined in the presence of eserine; nd, not done.

additional genes are probably involved (supporting online text). Our findings emphasize the importance of synaptic structure as a determinant of functional efficacy (*17*) and indicate that Dok-7–associated CMS should be classified as a synaptopathy rather than a channelopathy.

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19. Antiserum to Dok-7 was provided by Santa Cruz Biotechnology. We thank the CMS patients for participating in this study and B. Lecky, M. Roberts, T. Walls, D. Dick, U. Schara, U. Seifert, M. Hietala, M. Santos, J. Vilchez, and M. Shevell for patient referral. The work was supported by the UK Medical Research Council; the Muscular Dystrophy Campaign/Myasthenia Gravis Association of Great Britain; Grants in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan; grants from the Association Francaise contre les Myopathies; and the German Muscular Dystrophy Network, which is supported by the German Ministry of Education and Research.

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Functional Genomics

PUTTING GENES TO WORK

Many new technologies make genes more powerful than ever for basic and applied research. Advanced methods that turn gene expression up or down reveal what specific genes do and how to use that knowledge. Also, new approaches to informatics help scientists run more efficient experiments and gain more information from the data. **by Mike May and Gary Heebner**

Functional genomics covers a wide range of questions and techniques in biology. In addition, many of these areas grow rapidly, particularly as new technologies arise. In principle, this field addresses one question: What do genes do? Searching for that answer takes scientists from evolution to clinical applications. Moreover, approaching that question requires a variety of skills, including creating complete sets of DNA clones and sequences of genes from model organisms, studies in gene expression and control, and the development of experimental and computational methods for DNA and protein analyses.

To study the function of a single gene, scientists remove it from the parent organism and transfer it to a system where it can be studied in a controlled environment. With DNA cloning, for instance, a researcher isolates a gene and transfers it to another organism where it will replicate and be expressed as protein. In this way, a researcher can study a stretch of DNA and determine what it does. Several companies—such as **Clontech**, **EMD Biosciences**, **Epicentre Technologies**, **Lucigen**, and others—supply products for cloning, including kits that contain the necessary reagents.

Taking Over Expression

"Gene knockdown is the heart of functional genomics," says Mark Behlke, vice president of molecular genetics and biophysics at **Integrated DNA Technologies**. "The genome projects provide huge amounts of sequence data, and part of functional genomics is to dissect what these genes are and what role they play." A key approach to unraveling those roles involves turning genes on or off. By doing that, scientists can learn how particular genes impact downstream networks and what role they play in regulation.

Knocking down genes started about 30 years ago with antisense oligonucleotides. Behlke says, "These are single-stranded nucleic acids that are usually synthetic." The antisense oligonucleotides hybridize with target mRNA, which prevents gene expression. Antisense works through various methods, including RNase H mediated degradation. Many companies—including Integrated DNA Technologies, **Operon Biotechnologies**, **MoleculA**, and **R&D Systems**—provide antisense oligonucleotides.

In some cases, scientists control gene expression in intact organisms. In genetically engineered mice—often referred to as knockout mice—a particular gene sequence gets altered to keep it from being expressed. Large numbers of genetically altered mouse strains have been developed by companies like **Charles River Laboratories**, the **Jackson Laboratory**, and **Taconic**. In addition, **ArtisOptimus** grows primary mouse embryo fibroblasts (MEFs) that retain their initial growth and genetic properties, and this company can supply researchers with MEFs from a variety of knockout and transgenic mice.

An Explosion of Interference

Today, RNA interference (RNAi) makes up one of the most exciting areas in suppressing expression. Here, naturally occurring, double-stranded RNA—called small interfering RNA (siRNA)—inhibits the expression of a gene bearing its complementary sequence. This field also includes the use of microRNA (miRNA), which occur in nature as single-stranded nucleotides that are just 21 to 23 bases long. Some of these molecules can modulate gene expression by inhibiting translation or causing the degradation of mRNA. A number of companies—including **Ambion** (recently acquired by Applied Biosystems), **Dharmacon**, **Exiqon**, Integrated DNA Technologies, **Mirus Bio**, and others—provide kits and reagents for RNAi and miRNA studies.

"We have focused a lot of effort on our siRNA design tool," says Bettina Hädrich, development manager of gene silencing at **Qiagen**. "Our design algorithm and homology analysis are optimized to design siRNAs that are not only highly potent, but also highly specific so off-target effects can be avoided."

"The whole area of microRNA exploded over the past two years," says Peter Roberts, brand manager at Exiqon. "There are **continued ›**

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up to thousands of predicted microRNAs." In fact, hundreds have already been identified for vertebrates. Likewise, each miRNA could suppress dozens of targets. Roberts says, "Perturbed expression of microRNAs is implicated in a number of disease states." Imagine for example, a gene that codes for an apoptotic protein. If miRNA targeted to the expression of that protein gets overexpressed, the abundant miRNA could virtually eliminate the apoptotic protein. That could let some cells run wild, as in cancer.

To see if a particular miRNA is present, scientists can use commercially available kits, such as Exiqon's miRCURY LNA products. "This is used for detecting microRNA in cells and tissues," says Roberts. For instance, the July 8, 2005, *Science* includes a report by scientists from the Centre for Biomedical Genetics in the Netherlands, and they used Exiqon's technology to follow the expression of miRNAs in developing zebrafish.

Roberts says that scientists are already exploring miRNA for potential diagnostic applications. Overall, though, scientists still just want to know what miRNAs do. "But it's not just about figuring out what one microRNA does," says Roberts, "but figuring out how groups of different miRNAs act in concert to exert their influence."

At Integrated DNA Technologies, Behlke and his colleagues also see lots of basic research to come on RNAi. For example, this company directs many of its products at dicer, an enzyme that cleaves double-stranded RNA into siRNA. Lots of companies provide the products of dicer's work, the actual siRNA. Integrated DNA Technologies also offers dicer substrates. "It seems more potent to give the substrate rather than a product," says Behlke.

In addition, scientists at Integrated DNA Technologies design molecules that are predicted to be potent substrates for dicer. From that approach, they've been able to limit some off-target effects. This company also offers libraries. For example, customers can purchase four different dicer substrates related to 550 human kinases. Behlke says that one biotechnology company uses that kinome library to find hits related to cell growth. "They've found they are getting new hits that they'd missed with other libraries," says Behlke.

Advanced Control of Expression

RNAi can also be used in combination with other knockdown techniques. "Any of these techniques can lead to artifacts," says Behlke. "By trying more than one approach, you can make sure that the observed phenotype is real." For example, a scientist might use several siRNAs for the same target, and see if they create the same effect. Likewise, a scientist can turn off the same gene with RNAi and with antisense, and see if the two approaches yield the same result. Behlke says, "These are complementing technologies that are mutually supportive."

In the world of expression, scientists usually think of turning off genes, but it's also possible to turn on genes. "On the up-regulation side," says Behlke, "gene synthesis is becoming a new area, largely because the price has dropped dramatically." According to Behlke, gene synthesis used to cost about $15 per base, and now runs around $2 per base. Consequently, scientists can start to afford experiments in which they turn a gene off—with antisense or RNAi—and then add a synthetic gene that makes the same protein and see if that reinstates the normal phenotype.

Scientists can also learn more about genes by mutating them and looking for changes in expression. This is reverse engineered expression in a way. A scientist mutates a part of the system, and then studies how the system changes. Various companies—including **Finnzymes**, **New England Biolabs**, and **Sigma-Aldrich**—offer mutagenesis kits. These kits allow the selection of a desired mutation rate for a given sequence of DNA with a wide range of applicable DNA fragment sizes.

Taking on Transgenics

"Transgenic animals are an integral part of many functional genomics programs," says Oliver Franz, director of product management PCR, detection, and cell technology at **Eppendorf**. "They contribute to our understanding of developmental biology, behavior, oncogenesis, disease pathogenesis, and gene regulation." He adds, "Microinjection is one of the key steps in generating transgenic animals. It is needed to transport the foreign DNA into a targeted cell."

For microinjection, scientists can use Eppendorf's Femtojet. This electronically controlled device provides the pressure needed to deliver a sample solution—from femto- to microliters—from the microcapillary to the cell. Moreover, says Franz, "Its twin product, the Femtojet express, has been specifically designed for functional genomics applications since higher pressures can be applied by means of an external pressure source." He adds, "This way, a longer microinjection series at short intervals can be reliably performed."

Scientists often use the Eppendorf tools for pronuclear injection. "This is the standard technique to generate transgenic mice," says Franz. "A modified, linear DNA is introduced into the chromosomal DNA of fertilized eggs by injecting it through a fine glass microcapillary." Then, a scientist transfers the oocytes to the uterus of pseudopregnant animals. Finally, the offspring get screened for successful integration of the injected DNA. Franz says that Christian Klasen and his colleagues at EMBL in Heidelberg, Germany, often use the Femtojet for such injections.

Pushing Ahead with Proteins

Following the completion of many genome sequencing projects, a major focus of research is determining the structure and function of a large number of identified proteins. This requires fast access to proteins and multiparallel approaches. Jutta Drees, global product manager for protein expression and purification at Qiagen, says, "Recombinant proteins play an important role in functional genomics." For example, protein expression can be used to analyze

open reading frames to know if a protein is expressed or not. Protein expression also helps scientists study mutagenesis.

To keep track of all of the proteins being expressed, which can be thousands, scientists need fast and efficient techniques to express and deliver active forms of the proteins. "A number of applications need large amounts of really pure proteins, and scientists might need to label proteins, such as for structural studies, like NMR or crystallography. Our Ni-NTA offers researchers the best way to high-purity His-tagged proteins," says Drees. Typical in vivo approaches to expressing proteins, though, often take lots of time and need specialized equipment. "Conventional methods can take weeks," says Drees, "but our EasyXpress System is cell-free, rapid, and simple. It provides in vitro synthesis of large amounts of protein with or without labels in *E. coli* lysates." She adds, "This system contains all of the components for protein expression. You just add your DNA template, which can be a PCR product, and you can go from gene to protein in one day."

Drees says researchers use this system for many purposes. "It is often used in different screening systems," she says. "For example, it works well in mutational screening to study the function of proteins. It also works with studies of protein-protein interactions like pull-down studies." She adds, "Our system is also used in functional studies of newly identified proteins, such as enzymes."

In the future, Drees hopes to see new approaches that provide even higher yields of activity of in vitro expressed proteins. She also points out that the expression of some proteins—such as membrane proteins—proves challenging. She expects that new techniques will improve the ability to express these troublesome molecules.

Prowling in Packs

Typically, proteins function through pathways, which arise from interactions with other proteins. To identify related proteins, researchers often turn to the yeast two-hybrid method. This technique uses a transcription factor that binds to a gene and causes it to produce RNA and then protein. This transcription factor consists of two parts: the binding domain, which binds the gene; and the activation domain, which turns on RNA production. The domains get separated and fused to different proteins—ones that a researcher wants to test for interaction. If the two proteins do interact, the two domains will bind to each other and turn on RNA production. According to Ben Pricer, **Stratagene**'s product manager, "A two-hybrid system is often a starting point when trying to understand the role of a gene in downstream pathways." This system has been well developed in the yeast model and is offered by several companies, including **Invitrogen**, **OriGene Technologies**, and Stratagene.

Stratagene also offers BacterioMatch, which is a bacterial two-hybrid system. This system can explore endless questions. As Carsten Carstens, senior staff scientist at Stratagene, says, "Every function in cells is usually related to proteins interacting, and who interacts with whom is very important." In using BacterioMatch, researchers can test for interaction between two proteins. One gets attached to a repressor protein for a transcriptional operator, and the other is attached to a subunit of RNA polymerase. If the two proteins interact, it triggers transcription of the His3 reporter gene. Another reporter gene also gets expressed, for confirmation of the protein-protein interaction.

Some scientists use two-hybrid systems to screen libraries of proteins for interaction. "But instead of screening a library," Carstens says, "you will probably know if two proteins interact, and more often the quest is why the proteins interact." Here, interaction surfaces play a fundamental role. Scientists can create mutations in suspected binding areas and see if the interaction continues or stops. Carstens says, "This can be done much faster in a bacterial system."

Doubling the Density

"People have been doing gene expression for a while," says Kevin Meldrum, director of marketing at **Agilent**, "and most companies have built big databases, like ones that look at the cancer genome." He adds, "This gives more of a global view of gene expression, instead of gene by gene." Still, Meldrum hears from customers that they need a more mechanistic understanding of what genes do and how they do it. To help with that, Agilent developed its ChIP-on-chip (chromatin immunoprecipitation on a chip) microarrays.

These microarrays reveal how transcription factors interact with the genome. This company's latest products, which increase the density over past ones by almost six times, include 244,000 60-mer oligonucleotide probes. "The higher density allows more efficient experimental protocols, all at a lower price," says Meldrum. In the July 28, 2006, *Science*, Richard Young of the Whitehead Institute and his colleagues reported on experiments that used the new Agilent microarrays. The results revealed that protein kinases physically interact with the genes that they regulate.

Agilent will expand these higher density chips to new areas. Meldrum says, "We are already developing protocols on our chips that will explore miRNA. For example, you could use the miRNA as drug targets or as biomarkers for pharmacogenomics studies."

Enhancing the Workflow

"One of the biggest issues in functional genomics," says Darryl Gietzen, product manager for bioinformatics at **SciTegic**, a division of Accelrys, "is finding ways to best integrate all of the continued ›

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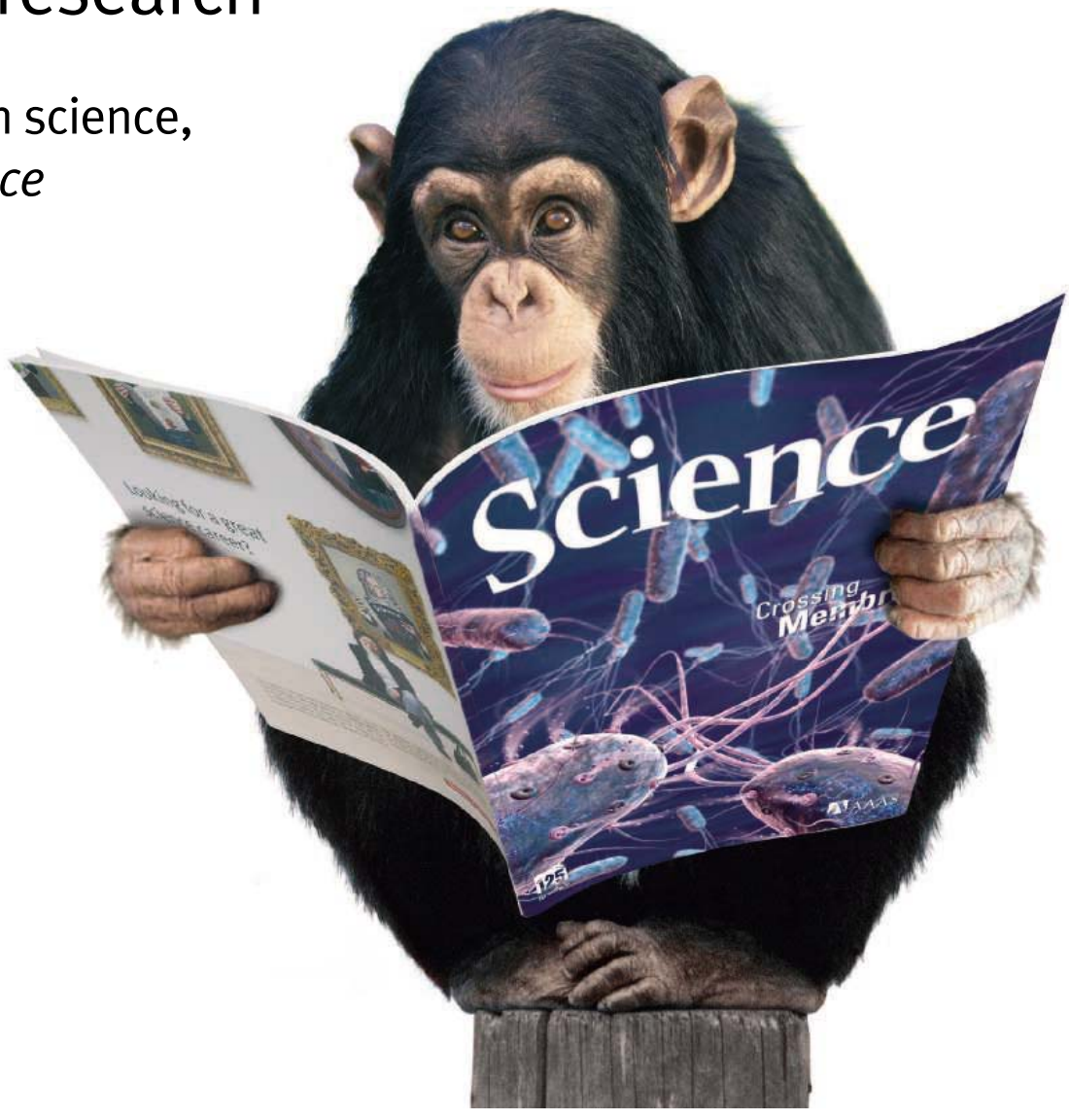

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available information." He adds, "Scientists want to gain knowledge from the information by bringing it together." Turning data into knowledge, though, takes the right bioinformatics. Several suppliers—including **DNASTAR**, **Elsevier MDL**, and SciTegic—offer suites of informatics tools that bring together data in a more meaningful platform.

To integrate data and algorithms, Gietzen and his colleagues offer Pipeline Pilot. With this software platform, a scientist can build an experimental, data processing workflow—essentially the step-by-step process of any experiment, from raw data through analysis to generating a final report. Moreover, Gietzen points out that researchers can modify an experimental protocol through Pipeline Pilot without involving a software developer.

Working with **Lucidyx**, Gietzen and his colleagues are also developing BioMining, a collection of modules for use in Pipeline Pilot. "BioMining offers one unified source of information," says Gietzen. "It allows someone to draw much better relationships between the data types and databases." He adds, "By integrating disparate data sources, scientists get much clearer answers to experimental questions." For example, Gietzen points out that a scientist with a list of interesting genes generated from mass spectroscopy data can gather available information—such as gene ontology, protein-protein interactions, and pathway information—and summarize it in an easy to understand, fully customizable report.

"In one view," says Gietzen, "a researcher can get a nice summary of information. We can condense a large amount of information into something that a human can interpret." Gietzen says researchers in pharma and biotech use Pipeline Pilot for many purposes, including designing siRNAs and antibodies. "The platform can be utilized almost anywhere," he says.

Still, many challenges lie ahead for functional genomics. For one thing, future software should help scientists use results from a past experiment to modify following studies. "We want scientists to be able to ask questions about data," says Gietzen, "and follow the scientific method of making an observation, generating a hypothesis, and then feeding that knowledge into future experiments to refine the hypothesis easily and quickly." Such an iterative approach—along with ongoing technological and theoretical advances—will push researchers ever closer to understanding just what genes do.

Mike May (mikemay@mindspring.com) is a publishing consultant for science and technology based in the state of Minnesota, U.S.A. Gary Heebner (gheebner@cell-associates.com) is a marketing consultant with Cell Associates in St. Louis, Missouri, U.S.A.

POSITIONS OPEN

FACULTY POSITION, ASSISTANT TO FULL PROFESSOR
University of Maryland School of Medicine

The interdepartmental Program in Genetics and Genomic Medicine at the University of Maryland School of Medicine is seeking candidates at the Assistant, Associate, and Professor ranks whose backgrounds and experience will support appointments on the tenure track. Qualified candidates should have expertise in human genetics, genetic epidemiology, molecular genetics (including model systems), statistical genetics, bioinformatics, pharmacogenomics, functional genomics, or related fields. These positions offer outstanding opportunities for independent and collaborative research in a highly interactive, multidisciplinary environment at the University of Maryland, Baltimore. Please send curriculum vitae and names of four references to: **Alan R. Shuldiner, M.D., Professor, Department of Medicine and Program Director, Genetics and Genomic Medicine, c/o JoAnn Gibbs, Department of Medicine, Room N3E10, University of Maryland Medical Center, 22 S. Greene Street, Baltimore, MD 21201.** Please cite positions 03-309-461/462.

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TENURE-TRACK FACULTY POSITIONS
Molecular Medicine and Genetics
Wayne State University School of Medicine

Multiple tenure-track positions at the **ASSISTANT, ASSOCIATE, or FULL PROFESSOR** levels are available as part of a multiyear hiring plan by the Center for Molecular Medicine and Genetics (CMMG). The Wayne State University (WSU) School of Medicine recently completed a $20 million renovation of the CMMG laboratory and core research facilities. To fill this new space, we are recruiting outstanding candidates holding Ph.D., M.D., or M.D.-Ph.D. degrees to join an active faculty conducting basic and translational research in systems biology, genomics, comparative genomics, neuroscience, computational biology, mitochondrial biology, and medical genetics. Joint appointments in clinical departments are available for Physician-Scientists. The CMMG **(website: http://www.genetics.wayne.edu)** offers extensive opportunities for collaboration both within the Center and throughout the University. Wayne State scientists have excellent opportunities to develop translational research with industry, government, and other academic institutions. Significant startup funds are available. Wayne State University is Michigan's only research University located in an urban setting. Applications will be reviewed upon receipt and continued until all positions have been filled.

Applications should include a letter of application, curriculum vitae, and the names and addresses of at least three references and be sent to: **Ms. Mary Anne Housey, Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Room 3127 Scott Hall, 540 E. Canfield Street, Detroit, MI 48201.**

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NIH-FUNDED POSTDOCTORAL POSITION. The project combines bioinformatic analysis with a forward genetic screen of *Drosophila* mutant fly lines to identify novel genes regulating feeding and fat deposition. Follow-up of corresponding mammalian homologs as candidate modulators of metabolic function will include biochemical and molecular pharmacological approaches in vitro and in vivo. Some knowledge of *Drosophila* is desirable, experience with molecular biology and *U.S. residency required*. Contact: **Alan S. Kopin, Director, Molecular Pharmacology Research Center; e-mail: akopin@tufts-nemc.org.** *Tufts New England Medical Center is an Equal Opportunity Employer.*

POSITIONS OPEN

BIOLOGY DEPARTMENT
University of North Dakota

The Department of Biology at the University of North Dakota invites applications for two tenure-track positions at the level of **ASSISTANT, ASSOCIATE, or FULL PROFESSOR.** A Ph.D. is required; postdoctoral experience is desirable. The successful candidates will establish productive, extramural-funded research programs that include the training of graduate students. Senior candidates should be leading a robust research program. Teaching expectations will not exceed two courses per year during the first several years. The Department offers graduate degrees through the Ph.D. and the campus environment is conducive for building a competitive research program. Genomics: The successful candidate uses genomics to address fundamental questions in biology and will strengthen an existing Departmental emphasis in molecular, cellular, and developmental biology. Teaching duties will include an undergraduate genomics course and an additional undergraduate offering. A graduate course will also eventually be expected. Quantitative wildlife biology: The position is integral to the Department's undergraduate degree in fisheries and wildlife biology and will also strengthen the Department's emphasis in ecology, evolution, and conservation biology. The successful candidate will combine field-based wildlife research with advanced statistical methods. We will consider scientists with a broad range of interests, but especially those with experience in waterfowl/grassland bird biology whose research will benefit by our location in the prairie pothole region of the northern Great Plains. Teaching duties will include undergraduate courses in wildlife management and ornithology, and an eventual graduate course in statistical analysis. Both positions will begin 16 August 2007. Review of applications will begin 27 October 2006, and continue until the position is filled. Send curriculum vitae, three representative reprints, statements of teaching and research interests, and three letters of reference to: **Dr. Peter Meberg** (genomics position) or **Dr. Rick Sweitzer** (wildlife position), **10 Cornell Street, Stop 9019, University of North Dakota, Grand Forks, ND 58202-9019.** For more information, see **website: http://www.und.edu/dept/biology/jobs.htm.** *The University of North Dakota is an Equal Opportunity/Affirmative Action Employer and we strongly encourage applications from women and underrepresented groups.*

Stephen F. Austin (SFA) State University invites applications for **CHAIR** of the Department of Biology to begin fall 2007. The successful applicant must have a Ph.D. in any area of biology with a strong record of scholarly activity and experience commensurate with the rank of tenured **FULL PROFESSOR** preferred, but highly qualified candidates at the rank of **ASSOCIATE PROFESSOR** will be considered. SFA seeks an individual who will provide innovative and energetic leadership with strong administrative skills and a demonstrated commitment to teaching, research, and faculty development. The successful applicant will be expected to teach courses in the successful candidate's field of expertise and possibly introductory courses. Additional information about the Department can be found at **website: http://www.sfasu.edu/biology,** and about the University at **website: http://www.sfasu.edu,** or contact: **Dr. Harry Downing** at **telephone: 936-468-3001,** or by **e-mail: hdowning@sfasu.edu.** Applicants should send curriculum vitae, the names and addresses of at least three references, and a cover letter addressing administrative style, teaching philosophy, and research plans to: **Chair Search, Attn: Harry Downing, Department of Biology, P.O. Box 13003, SFA Station, Nacogdoches, TX 75962.** Salary is commensurate with experience. Letters of recommendation will be required upon request. Review of applicants will begin immediately and continue until the position is filled. *SFA is an Equal Opportunity/Affirmative Action Education Institution and Employer. Security-sensitive position: criminal background check required for successful candidate. Applications subject to disclosure under Texas Open Records Act.*

Careers in Genetics and Genomics

Environmental Explorations

The advances in genotyping and functional genomics open new approaches to environmental science, including public health issues. A better understanding of epigenetics, for example, could reveal environmental triggers of cancer and other diseases. Here, experts in the field describe the most exciting advances in these areas and the best training to land a job. BY MIKE MAY

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The technologies behind today's genetics and genomics provide powerful tools for some obvious fields, such as medicine, but other fields can also benefit from these molecular capabilities. Environmental science looks particularly ripe for these applications. Genetic and genomic tools can enhance environmental research that ranges from traditional areas, like natural history and population genetics, to translational areas, such as public health. As a result, scientists who possess these tools can build careers in environmental research through many organizations.

First, how do genetics and genomics relate to environmental science? As one example of that relationship, Karl T. Kelsey, professor in the Department of Genetics and Complex Diseases at the Harvard School of Public Health, says, "In a broad sense, it is understanding exposures." But the tools for measuring exposure—say, to a dangerous compound or environment—still need work. Nonetheless, "genetics as an exposure tool will be a really exciting way to go," says Kelsey. Other tools—such as microarrays and proteomic applications—also improve the measurement of exposures. "Those tools, though, are in the early days," says Kelsey, "but that is where we are going."

Genomics can also be used to explore the structure of communities. For example, many fish stories—real ones—teach scientists more about the interaction between genes and the environment. Douglas Crawford, professor in the division of marine biology and fisheries at the University of Miami, studies fish of the genus *Fundulus*, which inhabit polluted coastal waters. In fact, these fish do better in polluted than clean water. The change in environment triggers changes in gene expression and even the physiology of these fishes. By looking at these natural populations, Crawford says that scientists can learn more about polymorphoric populations, instead of the inbred ones that grabbed most of the attention in the beginning of genomics. He says, "Natural populations include more genetic variation and recombination that puts together unique gene combinations, which show the complexity of organisms responding to environmental perturbations."

Putting Genes to Work

When asked about today's most exciting applications of genetics and genomics, Dabney K. Johnson, senior staff scientist and leader of the mammalian genetics and genomics group at Oak Ridge National Laboratory, says, "Oak Ridge is really at the forefront as far as mice are concerned, because we are generating a population of mice that is designed to represent in model form the genetic diversity that would be characteristic of a human population." (For more on these mice, see *Science* **301**:456–457, 2003.) She adds, "As this population progresses, these mice will be an important environmental resource." These mice will include 1,000 different genotypes. "You CONTINUED »

Careers in Genomics and Genetics


DOUGLAS CRAWFORD

could expose these different genotypes to environmental conditions of your choice," says Johnson.

The power of genetics and genomics also attracts environmental scientists who had never used these techniques. For example, George Roderick, professor of environmental science and curator of the Essig Museum of Entomology at the University of California, Berkeley, started as an ecologist but grew interested in using genes to understand more about ecology. As it turned out, he was entering a family business. In 1986, his father—Thomas H. Roderick of the Jackson Laboratory—came up with the word "genomics" to name a new journal. The name stuck—not only for the journal but for the field, as well.

"Genomics has opened up a huge range of new loci and what they do, especially in model organisms," says Roderick. "We have access to the adaptive nature of variation now." He sees that ability providing new approaches to a wide range of fields, including natural history. "In bacteria," he says, "there is excellent work showing how communities function and which genes are expressed when and how interactions occur."

High throughput screening of single nucleotide polymorphisms, or SNPs, can also reveal more of the genetic variation. "In out-bred species, like humans," says Crawford, "there's likely to be more than one solution to any environmental perturbations, but they will be genotype dependent."


DABNEY K. JOHNSON

Breeding Genetic Skills

Work like the Oak Ridge mouse project demands speed to create so many genomic lines. Johnson says, "High throughput genotyping is crucial." In addition, combining these genotypes with a variety of environmental conditions will generate huge volumes of data. "The analytical techniques—bioinformatics—will also be crucial," says Johnson, "to pull out the subtle but vital patterns and correlations from this vast swamp of data."

Instead of just seeing how genes impact the phenotype, scientists can also explore how the environment impacts the genes. This field—called epigenetics—could teach scientists even more about the environmental influence on diseases. "One really exciting area is the effect of the environment on chromatin," says Kelsey. "The techniques for looking at this are growing fast," he says. They will include microarrays, microRNA, and all of the new noncoding RNAs.

Today's students can get the tools that they need to approach environmental questions with genetics and genomics. For example, the University of California, Berkeley, offers a major in molecular environmental biology. Roderick says, "This program draws lots of students who want to do something in environmental sciences and use modern molecular skills." He adds that these students usually know a lot about lab skills, but can lack basic background, like a general understanding of biology, evolutionary biology, and natural history.


GEORGE RODERICK

A Consensus on Computation

Success in the future will depend on analytical skills. "Taking on this huge mass of data will require supercomputers," Johnson says. "It is no longer a workstation kind of analysis." Moreover, a scientist must know or develop ways to find the smallest differences. "How in the world do you find the patterns and correlations that are real and make the difference between a smoker who gets lung cancer and one who does not?" Johnson asks. "You need to find correlations between single base-pair differences in the genome and how healthy or unhealthy people have lived." The scientists who can find those correlations will also find great jobs.

Kelsey agrees that the future will demand strong analytical skills. He mentions biostatistics and epidemiology. In addition, his students learn programming. "I believe they need that to go forward," he says. "If you can't program, you'll be left behind." The computational requirements will also continue to grow in this field. Kelsey says, "You must run the software and understand how people think about it." In thinking about all of the computations being done and the ones that will be done soon, he pauses and then adds, "It's a very fast-moving field. It's extremely fun!"

For working on animals from the natural environment, Crawford recommends a knowledge of statistics and population genetics, as well as an understanding of molecular biology and how the available techniques work. The real key, he says, is finding ways to precisely and repeatedly quantify small variations. As he points out, "Michael Jordan does not jump twice as high as me, and Tiger Woods does not drive a golf ball twice as far as me, but who do you want on your team?" Although he adds that Tiger can out drive him by 100 yards, he says that small differences could mean everything. So scientists must develop accurate techniques that consistently reveal small variations in the interactions between genes and the environment.

A Growing Future

In the near future, Roderick expects the availability of loci to extend beyond model organisms and ones with relatively small genomes. "Being able to make use of that information in relevant ways will be a big field," he says. "The ability to make use of this information is an area that is bound to grow."

Just in case the quantitative angle of this article was not emphasized enough, remember this: "The future will be dense data," says Kelsey. So set up a keyboard and start calculating a path to a career that unravels the environment with genetics and genomics.

Mike May (mikemay@mindspring.com) is a publishing consultant for science and technology based in the state of Minnesota, U.S.A.

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

CAMPUS-WIDE FACULTY HIRING INITIATIVE IN EVOLUTIONARY GENOMICS

The University of Illinois at Urbana-Champaign is devoting significant new resources to the further strengthening of its programs in genomic biology and related disciplines. The Institute for Genomic Biology is co-sponsoring five new tenure-track faculty positions in the area of Evolutionary Genomics. Faculty hiring will be based in one or more academic departments, with the possibility of a joint appointment in an Institute for Genomic Biology research theme (http://www.igb.uiuc.edu/research.html). As established leaders in the genomics of model organisms and integrative biology, the University seeks ambitious scientists who wish to work in an unparalleled interdisciplinary environment to pursue fundamental questions in evolutionary biology using genomic approaches.

THE DEPARTMENT OF ANIMAL BIOLOGY invites applications for a full-time (9-month), tenure-track faculty position at the assistant professor level to begin August 16, 2007, or negotiable. We seek candidates who can establish a vigorous, externally funded research program in vertebrate evolutionary-developmental biology, broadly construed. Areas of interest include, but are not limited to, evolution of phenotypic variation or plasticity and genomic studies of selection or diversification. We are particularly interested in candidates using or developing genomic approaches. The successful candidate will be expected to collaborate with current faculty to develop major research initiatives in evolutionary genomics. For full consideration, applications must be received by December 8, 2006. For more information see www.life.uiuc.edu/sib, email sib@life.uiuc.edu, or call 217.333.3044.

THE DEPARTMENT OF ANIMAL SCIENCES is searching for a full-time (9-month), tenure-track assistant professor in evolutionary genomics. The successful candidate will contribute to departmental and campus-wide programs in evolutionary and statistical genomics, and bioinformatics. The successful candidate's research program will utilize genomic tools to address questions relating to the evolution of vertebrate genomes, the genomic basis of adaptive evolutionary responses, and genomic changes resulting from intensive selection and domestication. Questions should be directed to Dr. Lawrence B. Schook at 217.265.5326 or schook@uiuc.edu. Applications received by October 31, 2006, will receive full consideration. Further application information is detailed at www.ansci.uiuc.edu/jobs/searches/evo_genomics/.

THE DEPARTMENTS OF ENTOMOLOGY AND PATHOBIOLOGY seek an outstanding scientist with a background in interdisciplinary research involving the evolution of arthropod vector-pathogen interactions, genetics and genomics, for a full-time, tenured faculty position at the Associate Professor or Professor level to begin August 2007, or as negotiated. Candidates must have a Ph.D. in a relevant field, such as Molecular Evolution, Entomology, Microbiology, Parasitology or Genetics. The successful candidate will have developed an externally funded research program involving genome-wide studies to investigate the origin and evolution of genes essential to the establishment and maintenance of arthropod vector-pathogen interactions. For full consideration, applications must be received by December 8, 2006. For more information, see www.life.uiuc.edu/sib and www.cvm.uiuc.edu/vp, email derosset@uiuc.edu, or call 217.333.2449.

THE DEPARTMENT OF NATURAL RESOURCES AND ENVIRONMENTAL SCIENCES seeks a full-time (9-month), tenure-track assistant professor of plant evolutionary genomics. Applicants should have a Ph.D. and a multidisciplinary background that combines plant evolution and genetics, molecular biology, and functional genomics. We prefer candidates who can apply genomics tools to study genetic patterns of perennial plant evolution in response to artificial selection and domestication, and who will develop an internationally recognized research program, direct graduate students, interact with undergraduates, contribute to teaching needs, and compete for research funds. For full consideration, applications must be received by October 16, 2006. For more information, see www.nres.uiuc.edu, or call 217.333.9738.

THE DEPARTMENT OF PSYCHOLOGY is searching for a full-time (9-month) faculty member with expertise in the area of molecular genetics and individual differences in human behavior, broadly construed. We are particularly interested in applications from scholars who study the biological and genetic epidemiological systems that contribute to psychopathology, cognitive development, social behavior, and personality. Further application information, including other faculty openings, closing application date, and starting employment dates, is detailed at www.psych.uiuc.edu. For more information, call 217.333.2644, or contact broberts@cyrus.psych.uiuc.edu.

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Computational Biology and Genome Analysis Faculty Positions

Department of Genetics
Washington University Medical School

The Department of Genetics at the Washington University School of Medicine is planning to hire new faculty in the general area of Computational Biology and Genome Analysis. Applications are invited at all levels, from Assistant Professor to Full Professor. The Department currently has strengths in the areas of algorithms for sequence analysis and modeling of regulatory networks. Applicants working in these areas are encouraged to apply, as well as in other areas that would complement our current strengths. The Department is also the home to many outstanding laboratories involved in basic research in model organisms and human genetics.

Washington University is an excellent environment for conducting Computational Biology and Genomics research. It has a renowned Graduate Program in Computational Biology that includes faculty from several departments who are doing research in many areas. It is the home of the Washington University Genome Sequencing Center that has been at the forefront of large scale DNA sequence efforts for many years and contributed significantly to the human genome sequence and those of many other species. Washington University is also the home to the Center for Computational Biology and the newly formed Center for Genome Sciences, both of which bring together faculty from multiple departments with shared interests in Computational Biology and Genomics research.

All applications received before **December 1, 2006**, will be considered. Applicants should send, either by email (preferred) or regular mail, a current CV, a statement of research interests, and arrange to have three letters of recommendation sent to: **Dr. Gary Stormo, Erlanger Professor, Department of Genetics, Director, Computational Biology Program, Washington University Medical School, 4444 Forest Park Ave, CAMP BOX 8510, ST. LOUIS, MO 63108-8510; email: stormo@genetics.wustl.edu.**

Women and members of minority groups are encouraged to apply. Washington University is an Affirmative Action Employer.

Center for Diabetes Research
Center for Human Genomics
Wake Forest University School of Medicine

Faculty Positions

Applications are invited for tenure track positions at the Assistant or Associate Professor level. The successful candidates will join an active, well-funded group of interdisciplinary researchers with special strengths in genetics and population-based research with state-of-the-art facilities and active graduate and postdoctoral training programs. Positions are committed to building the area of basic research in diabetes, obesity, and metabolism and expanding molecular genetics. Investigators that bring novel technical approaches to an interdisciplinary environment are especially encouraged.

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Genetic Analysis and Bioinformatics

Experience in modern approaches for genetic analysis of complex traits in human systems and integration with informatic tools. A professional focus of participating in mapping and identification of human disease genes is essential.

Contact: Applicants should send curriculum vitae, statement of research interests, and the names of 3 references to: **Donald W. Bowden, Ph.D., Director, Diabetes Research Center, Wake Forest University School of Medicine, Medical Center Blvd., Winston-Salem, NC 27157. Email: dbowden@wfubmc.edu**.

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Interviews will be held in Strasbourg the week of October 16.

For information on all ESF activities, please go to www.esf.org

CAREERS IN GENETICS & GENOMICS

FLORIDA STATE UNIVERSITY

DEPARTMENT OF BIOLOGICAL SCIENCE
EIGHT TENURE-TRACK FACULTY POSITIONS
INTEGRATING GENOTYPE AND PHENOTYPE

The Department of Biological Science invites applications from molecular and evolutionary geneticists working to understand the genotype-phenotype map. Eight tenure-track positions at all ranks will be filled over the next few years. Two types of applications are being solicited: (1) individual candidates seeking the challenge of helping build an interactive group of faculty and, (2) groups of candidates at any level whose research collectively fits the mission of the initiative. Emphasis will be placed on hiring groups or individuals exploring gene regulatory mechanisms and their consequences for phenotypic variation and evolutionary change. Areas of particular interest include epigenetics, RNA interactions, chromatin biology, comparative genomics, quantitative genetics, and developmental biology. This initiative coincides with the construction of a new Life Science Research and Teaching Building and is part of the Pathways of Excellence Program aimed at propelling FSU into the top rank of public universities.

To apply, please submit electronic copies (PDF files preferred) of a cover letter, curriculum vitae, statements of research and teaching interests, and the names and email addresses of four references to **Dr. David Houle, Chair, Genotype-Phenotype Search Committee, email: genphensearch@bio.fsu.edu**. Group applications should, in addition, identify a primary contact person and provide a brief description of the integrative nature of the group and the anticipated collaborative interactions among the constituent members. Informal inquiries about submitting group applications are encouraged prior to submission. For detailed information please visit: **http://www.bio.fsu.edu/genphensearch/**. Review of applications will begin **December 8, 2006**.

FSU is an AA/EO Employer. Applications from minority and female candidates are especially encouraged.

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Imagine a career that touches the lives of people everywhere. Imagine an opportunity to reach beyond your area of expertise to make an impact on something greater than the bottom line. Imagine playing a key role in some of the most critical issues facing healthcare today. This is your career at Pfizer - a career unlike any other.

At our state-of-the-art laboratories in La Jolla, the Departments of Biochemical Pharmacology and Structural and Computational Biology & Design are committed to excellence in science and the discovery of innovative pharmaceuticals. We have a strong team of highly motivated and skilled research scientists with an established history of applying mechanistic enzymology, biophysics, and structure-based drug design to the discovery of novel therapeutic agents. We are looking for highly skilled scientists who share our vision and enthusiasm for drug discovery to apply for the following open positions:

Biophysicist, PhD-Level

Requires 3+ years of experience in biophysical molecular interactions and protein characterization with expertise in calorimetry, SPR, and protein mass spectroscopy preferred. **Req# 055588**

Structural Biologist, PhD Level
Principal/Senior Principal Scientist

Requires 6+ years experience in construct design, expression, purification, characterization and crystallization of recombinant proteins to drive x-ray crystallography. Significant managerial experience is also required. **Req# 057566**

We offer competitive compensation, full benefits and talented professional colleagues…some of the best and brightest in the research field today. Submit your resume today at: **www.pfizer.com/careers** and search by the appropriate Req number.

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Positions @ NIH
THE NATIONAL INSTITUTES OF HEALTH

Look into INRO
The Intramural NIAID Research Opportunities Program

Help Today's Science Student Become Tomorrow's Top Researcher

The National Institute of Allergy and Infectious Diseases (NIAID), Division of Intramural Research (DIR), is seeking applicants for its Intramural NIAID Research Opportunities (INRO) program. INRO, currently in its fifth year, is a 5-day exploratory program intended for underrepresented minority students (i.e., American Indians or Alaska Natives, Native Hawaiians or other Pacific Islanders, blacks or African Americans, and Hispanics or Latinos) who are interested in a research career in the areas of allergy, immunology, and infectious diseases.

This 5-day program will be held February 4–8, 2007, at the National Institutes of Health in Bethesda, Maryland. Applicants to this highly competitive program should be undergraduate juniors or seniors interested in postbaccalaureate biomedical research, doctoral candidates seeking a postdoctoral training position, and first-year medical students contemplating a year-off research position.

Students selected to attend the INRO 2007 program will:

• Learn about the Institute and about training opportunities available at NIAID • Listen to scientific lectures by world-renowned NIAID researchers • Tour Institute laboratories • Interview with principal investigators for potential training positions

The INRO program will pay all expenses for travel, hotel accommodations, and meals. Eligible students include U.S. citizens or legal U.S. residents who belong to a minority population that is underrepresented in the sciences. Only students with strong academic standing will be considered.

For more details about student eligibility, highlights of last year's INRO program agenda, student testimonials from past programs, and detailed information about the application process, students may visit our Web site at www.niaid.nih.gov/labs/training/inro. Completed applications MUST be received by **October 15, 2006**.

For additional information, contact **Wendy J. Fibison, Ph.D., Associate Director, DIR, NIAID, wfibison@niaid.nih.gov.**

TENURE-TRACK INVESTIGATOR, NICHD

The Program on Cell Regulation and Metabolism (PCRM), National Institute of Child Health and Human Development, invites applicants for a Tenure-track Principal Investigator studying gene expression, cell cycle control, signal transduction, or development using yeast, fruit fly, or nematode as a model system and emphasizing a genetic approach. The PCRM is an interactive environment featuring studies on transcriptional and translational control of gene expression, signal transduction, chromosome structure, nucleocytoplasmic transport, cell cycle control, transposable elements, DNA repair, hormone regulation of development, and development of the visual system using bacteria, yeast, fruit fly, frog, and mammalian cells as experimental systems. Applicants should have a Ph.D. or M.D. and a proven ability to conduct innovative research. Send a c.v., 2-page statement of research plans, and 3 recommendation letters by **November 15, 2006** to: **Alan Hinnebusch, Ph.D., NIH, Bldg 18T, Rm 106, Bethesda, MD 20892.**

NATIONAL CANCER INSTITUTE

HIV and AIDS Malignancy Branch
Center for Cancer Research

Tenured/Tenure Track Position
Translational Researcher in Viral Oncogenesis

The HIV and AIDS Malignancy Branch (HAMB), NCI, is searching for a tenure-track or tenured investigator in the field of viral oncogenesis. It is anticipated that the investigator will establish an independent research program targeted to the study of the treatment, pathogenesis, and/or prevention of viral-induced tumors, especially those associated with AIDS. The research program should be translational in focus and be able to interface with a strong existing clinical research program in AIDS-related tumors. HAMB is located on the Bethesda campus of the NIH (http://ccr.cancer.gov/labs/lab.asp?labid=63). Current areas of research in HAMB focus on Kaposi's sarcoma-associated herpesvirus (KSHV /HHV -8)-associated tumors, the molecular biology of human papillomavirus (HPV), and the development of novel therapeutic interventions for HIV infection. Candidates for the position should have an M.D./Ph.D., Ph.D., or M.D. and strong research credentials. Applicants for this position should submit a curriculum vitae including bibliography, a statement of research interests, a two-page outline of the proposed research program, and the names of three references to **Chairman, Search Committee, HAMB, NCI, Attention Jan Huque, Building 10, Rm.10S255, 10 Center Drive, M.S.C. 1868, Bethesda, MD 20892-1868 no later than December 7, 2006**. You may also e-mail your application to: **huquei@mai1.nih.gov (Jan Huque, 301-435-4627)**.

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CANCER DIAGNOSIS PROGRAM
PROGRAM DIRECTOR
NATIONAL INSTITUTES OF HEALTH
NATIONAL CANCER INSTITUTE

The Cancer Diagnosis Program (CDP) is an extramural program within the Division of Cancer Treatment and Diagnosis of the National Cancer Institute (NCI) responsible for facilitating the translation of new knowledge in cancer biology and technologies into clinically useful diagnostic and predictive tests. CDP initiated the Program for the Assessment of Clinical Cancer Tests (PACCT) in 2000 to ensure that the next generation of biomarkers and laboratory tests improve the management of cancer patients. CDP works closely with other NCI units and with other government agencies that focus on related aspects of the diagnosis challenge. These include the Cancer Therapy Evaluation Program (CTEP), responsible for the NCI's clinical trials program; the Cancer Imaging Program, responsible for improvements in the non-invasive imaging of tumor physiology and biochemistry; staff from various programs involved in the development of state-of-the art informatics systems, and statistical and mathematical techniques adequate for the analysis of massive datasets; other components of the NCI; the National Institute of Standards and Technology; and such regulatory agencies as the FDA. Since the movement of new diagnostic and predictive tests into clinical practice also depends on interactions with the international oncology community, CDP also fosters collaborations with foreign oncology groups.

CDP is seeking an M.D., Ph.D. or D.O. to serve as a Program Director in the Diagnostics Evaluation Branch (DEB) to participate in a dynamic extramural research program of international scope. Experience with clinical trials and an interest in diagnosis and/or predicting the response to treatment, particularly as it relates to evaluation of biomarkers and in vitro diagnostic tools is necessary. The Program seeks an individual with experience in the translation of new knowledge and technology to clinical practice. A knowledge of systems biology and bioinformatics especially as it relates to identification of biomarkers or groups of biomarkers is helpful. Also helpful is experience that involves understanding the clinical decisions that can be informed by the use of markers and molecular technologies. The candidate will work with the Chief of the Diagnostics Evaluation Branch of the CDP and staff in the development of new initiatives for both the academic and business research communities. Significant effort will be devoted to projects initiated as part of PACCT.

Base salary for this position ranges from $91,407 to $118,828 per annum. MD and DO candidates are eligible for an additional allowance beginning at $13,000 per annum, depending on qualifications. Benefits include health and life insurance options, retirement, paid holidays and vacation leave.

To apply for this position, please visit: **http://jobsearch.usajobs.opm.gov/a9nih.asp** and keyword search for Vacancy Announcements **(VA), NCI-06-142673 (Ph.D.) or NCI-06-142674-DH (MD or DO)** for the mandatory application requirements. You must apply by the closing date of **October 30, 2006**. For questions about applying to the VA, please contact **Mary Lou Weathers, on (301) 402-5059 or weatherm@mail.nih.gov**.

For more information about the position, please contact **J. Milburn Jessup, MD at jessupj@mail.nih.gov or (301) 435-9010**.

Department of Health and Human Services
National Institutes of Health
National Cancer Institute
Tenured, Tenure-Track Investigator

With nationwide responsibility for improving the health and well being of all Americans, the Department of Health and Human Services (DHHS) oversees the biomedical research programs of the National Institutes of Health (NIH) and its research Institutes.

The National Cancer Institute (NCI), a major component of the NIH, is recruiting a tenure-track or tenured investigator to conduct primary research focused on human cancer risk related to infection or to perturbation of immunity or inflammation. The successful candidate will work in the Viral Epidemiology Branch, Division of Cancer Epidemiology and Genetics, NCI. Typical research projects are national or international in scope and involve collaborations with statisticians, clinicians, virologists, immunologists, geneticists, and other scientists throughout NIH and extramurally.

Candidates must have an M.D., Ph.D., or equivalent doctoral degree. They must have substantial experience in human viral, bacterial or cancer epidemiology. Demonstrated experience with the design, conduct, and analysis of epidemiologic studies is required. Candidates must have a sound working knowledge of basic statistical methods. Demonstrated experience with analyses of large databases, genomics, or genetic polymorphisms would be advantageous, as would clinical or laboratory skills. Candidates must have displayed strong leadership in independent, innovative research. They also should have outstanding communication skills for writing research papers, presenting at scientific meetings, and collaborating with scientists of various backgrounds. A substantial record of relevant publications in the peer-reviewed medical or scientific literature is required.

Applicants should send a cover letter that briefly summarizes their research interests and experience and future goals; curriculum vitae with bibliography; copies of three selected publications; and three letters of reference to: **Chair, Search Committee, Viral Epidemiology Branch, NCI, c/o Mrs. Judy Schwadron, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Executive Plaza South, Room 8073, Bethesda, MD 20892, Or via email to: jrussell@mail.nih.gov.**

Candidates should submit applications by November 15, 2006, however, the search will continue until a qualified applicant is found.

Positions @ NIH
THE NATIONAL INSTITUTES OF HEALTH

Chief, Laboratory of Bacterial Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health

The National Institute of Allergy & Infectious Diseases (NIAID), Division of Intramural Research (DIR) is seeking an outstanding individual to head the newly established Laboratory of Bacterial Diseases (LBD) in Bethesda, Maryland. The laboratory is to be located in the new C.W. Bill Young Center for Biodefense and Emerging Pathogens located on the NIH campus in Bethesda, Maryland.

The mission of the LBD will be to study basic and applied aspects of bacterial diseases related to biodefense or emerging and re-emerging pathogens, focusing on pathogenic bacteria. Exceptional scientists with research interests in basic, translational or clinical aspects of bacterial pathogenesis are encouraged to apply. The long-term goals of the Institute include supporting research that enables the development of new diagnostics, vaccines, and therapeutics.

This position requires an M.D., Ph.D. or equivalent with proven leadership abilities and a strong independent research program. Preference will be given to candidates with a documented record of accomplishment in bacterial disease research, and those whose program(s) are consistent with the mission of the NIAID.

The Laboratory Chief will have independent resources to conduct basic and clinical research and will supervise other Principal Investigators with independent research programs. The successful candidate is expected to lead a strong research program in laboratory and/or clinical research. Committed resources include space, support personnel and an allocated annual budget to cover service, supplies, animals and related resources and salaries. A Laboratory Chief in the DIR is equivalent to a Department Chair in a University or Medical School. Applicants must be U.S. citizens or permanent residents and be eligible for the appropriate security clearance under the CDC Select Agent Program. Salary will be commensurate with experience and qualifications.

Interested candidates may contact **Dr. Karyl Barron, Deputy Director, DIR, NIAID at 301/402-2208 or email (kbarron@niaid.nih.gov)** for additional information about the position and/or infectious diseases research at the NIH.

To apply for the position, candidates must submit curriculum vitae, bibliography, a detailed statement of research interests, and reprints of up to three selected publications, preferably via Email to: Lynn Novelli at novelly@niaid.nih.gov. In addition, the names of three potential references must be sent to **Dr. Steven M. Holland, Chair, NIAID Search Committee, c/o Ms. Lynn Novelli, DIR Committee Manager, 10 Center Drive, MSC 1356, Building 10, Room 4A26, Bethesda, Maryland 20892-1356.** Completed applications **MUST** be received by **Friday, November 3, 2006**. Please refer to **AD#004** on all correspondence. Further information on this position and guidance on submitting your application is available on our website at: **http://healthresearch.niaid.nih.gov**

The National Institute of Allergy and Infectious Diseases, a major research component of the NIH and the Department of Health and Human Services, is recruiting a Staff Scientist. The position will be available in the Respiratory Viruses Section of the Laboratory of Infectious Diseases, and scientists with a M.D., D.V.M, or Ph.D. are eligible. The research activity involves (1) examination of the pathogenesis of pandemic and potential pandemic strains of influenza and their evaluation in vitro and in experimental animals; (2) influenza viral genomics, and examination of viral evolution in fitness and host adaptation; and (3) the development of influenza clinical trials in humans. This full-time research position offers a unique opportunity to work on investigations that range from basic molecular biology to clinical research. Staff Scientist applicants should have at least six years of laboratory work experience in molecular and classical virology research; the salary range is $73,178 - $165,195. Preference will be given to candidates who have experience working with avian influenza viruses and those with BSL3 experience. Applicants should submit their curriculum vitae, a letter of research interests, and names and addresses of three references to:**Jeffery K. Taubenberger, MD, PhD, Attn: D. Kyle, NIAID, NIH, Bldg 50 Room 6234, MSC 8007, 50 South Drive, Bethesda, MD 20892-8007, FAX: (301) 496-8312, email: dkyle@niaid.nih.gov**

Review of applicants will begin on **October 30, 2006** and continue until a successful candidate is identified.

The National Institute of Allergy and Infectious Diseases, a major research component of the NIH and the Department of Health and Human Services, is recruiting a Staff Scientist. The position will be available in the Respiratory Viruses Section of the Laboratory of Infectious Diseases, and scientists with a M.D., D.V.M, or Ph.D. are eligible. The research activity involves (1) the development of live attenuated vaccines against potential pandemic strains of influenza and their evaluation in experimental animals as well as in clinical trials in humans; (2) examination of the pathogenesis of avian influenza viruses and SARS-coronavirus; (3) the evaluation of the immunologic determinants of resistance to infection and disease caused by influenza viruses and SARS-coronavirus. This full-time research position offers a unique opportunity to work on investigations that range from basic molecular biology to applied vaccinology. Staff Scientist applicants should have at least six years of laboratory work experience in molecular virology and vaccine research; the salary range is $73,178 - $165,195. Preference will be given to candidates who have experience working with avian influenza viruses. Applicants should submit their curriculum vitae, a letter of research interests, and names and addresses of three references to: **Kanta Subbarao, MD, MPH, Attn: A. LeCointe, NIAID, NIH, Bldg 50 Room 6234, MSC 8007, 50 South Drive, Bethesda, MD 20892-8007, FAX: (301) 496-8312, email: lecointe@niaid.nih.gov**

Review of applicants will begin on **October 15, 200**6 and continue until a successful candidate is identified.

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Department of Health and Human Services
National Institutes of Health
National Institute of Allergy and Infectious Diseases

With nation-wide responsibility for improving the health and well being of all Americans, the Department of Health and Human Services oversees the biomedical research programs of the National Institutes of Health (NIH) and those of NIH's research Institutes.

The National Institute of Allergy and Infectious Diseases (NIAID), a major research component of the NIH and the Department of Health and Human Services, is recruiting for a Tenure/Tenure Track position in the Laboratory of Host Defenses (LHD). The LHD studies immune functions essential for host defense against infection (inherited immune deficiencies) and those required for immune homeostasis (autoimmunity associated with excessive inflammation). The LHD seeks an M.D. or M.D., Ph.D. physician scientist to develop an independent translational research program related to the genetic basis, pathophysiology, diagnosis and treatment of autoimmune diseases associated with excessive inflammation. An emphasis on clinical aspects of innate immunity including phagocytic cells, natural killer cells, dendritic cells and other antigen presenting cells, toll-like receptors or other pattern recognition receptors in its interface with acquired immunity is desirable. The applicant should have a strong track record of basic research of the genetic basis of disease and alterations in signaling pathways responsible for immune dysregulation. The applicant must possess expertise and experience in the design and conduct of diagnostic and therapeutic clinical trials studying and treating autoimmune diseases. Strong clinical credentials in a specialty area relevant to the proposed translational research program (relevant specialties include but are not limited to rheumatology, pulmonary diseases, hematology, immunology or infectious diseases) are required. The program of study proposed by the applicant must include both laboratory components and the conduct of clinical protocols to assess new diagnostic and therapeutic modalities to diagnose and treat autoimmunity associated with excessive inflammation. Applicants particularly suitable for this program are those who have knowledge and experience in the development and clinical application of novel biological agents including chemokines, soluble chemokine receptors, adenosine receptor agonists, monoclonal antibodies, cellular therapies including transplantation or gene therapy to correct the abnormalities in immunity, that achieve immune tolerance or to reduce abnormal inflammation.

The applicant must provide evidence in the submitted materials that the applicant has a current license to practice medicine in one of the states of the United States or must have all the credentials required by the State of Maryland for licensing to allow the practice of medicine. These credentials must include but are not limited to having a Doctor of Medicine or Doctor of Osteopathy degree from an accredited school in the U.S. or Canada, or a Doctor of Medicine or equivalent degree from a foreign medical school that provided education and medical knowledge substantially equivalent to accredited schools in the U.S. as demonstrated by permanent certification by the Educational Commission for Foreign Medical Graduates (ECFMG).

To be considered for this position, you will need to submit a curriculum vitae, bibliography, three (3) letters of reference, a detailed statement of research interests, and a hardcopy of selected publications to **Thomas A. Fleisher, MD, Chairperson, NIAID Search Committee, c/o Ms. Anissa N. Hunter, DIR Committee Coordinator, Reference Ad #009, 10 Center Drive MSC 1356, Building 10, Rm. 4A26, Bethesda, Maryland 20892-1356**. Completed applications **MUST** be received by **Thursday, November 15, 2006**. For additional information on this position, and for instructions on submitting your application, please see our website at: **www.niaid.nih.gov**.

Positions @ NIH
THE NATIONAL INSTITUTES OF HEALTH

Department of Health & Human Services (DHHS)
National Institutes of Health (NIH)
National Institute of Dental and Craniofacial Research (NIDCR)

The National Institute of Dental and Craniofacial Research (NIDCR), National Institutes of Health (NIH), Department of Health & Human Services (DHHS) is seeking applicants for a Biologist/Microbiologist/Health Scientist Administrator position in the Center for Integrative Biology and Infectious Diseases (CIBID). The position advertised is for the Director of the Microbiology Program. This program supports extramural basic and translational research on the role of oral microbes in health and disease. To this end, four broad scientific areas provide the basis for rapid development of knowledge of the etiology, pathogenesis, diagnosis, treatment and prevention of oral infectious diseases. These interrelated areas are: (i) Biofilms and Microbial Ecology; (ii) Microbial genomics; (iii) Microbial Virulence and Disease Pathogenesis; and (iv) Prevention and Treatment.

The incumbent will direct, administer and evaluate a portfolio of extramural grants, contracts and cooperative agreements and will stimulate interest in and provide advice to the extramural community regarding the respective research portfolio. In addition, the incumbent will participate in funding decisions, policy development, as well as implementation and coordination with other programs both within and outside of the NIDCR.

The salary range for this position is $77,353 to $118,828 per annum, commensurate with qualifications and professional experience. A full benefits package is available, which includes retirement, Thrift Savings Plan participation, health, life and long-term care insurance.

For qualifications required, evaluation criteria, and application instructions, view the vacancy announcements at: **http://jobsearch.usajobs.opm.gov/a9nih.asp**. Refer to announcement **# NIDCR-06-141634DE** or **NIDCR-06-147841MP**. Applications will be accepted until **October 27, 2006**. Please contact **Michelle Lipinski at 301-594-2286** or **lipinskim@od.nih.gov** if you have questions.

TenureTrack/Tenure Position
Neurovirology
Division of Intramural Research

The Division of Intramural Research of the National Institute of Neurological Disorders and Stroke is recruiting an individual for a tenure track position in the area of neurovirology with special interests and expertise in viruses that target the brain. Recruitment for a senior individual for a tenured position would also be considered. The individual would direct an independent research program on the molecular, biological, immunological, and/or clinical aspects of the neurological complications of viral infections of the human nervous system which could include HIV-1. The program would conduct its work in conjunction with the Laboratory of Molecular Medicine and Neuroscience which has established areas of research on the infectious etiology of neurodegenerative diseases. The individual would have a demonstrated background and knowledge in virus-cell interactions using molecular technologies to investigate multifactorial mechanisms of damage to the brain including viral proteins, inflammatory molecules and chemokines. Experience with animal models of viral infections in the brain would be desirable. The candidate will have an earned Ph.D. and/or M.D. degree with excellent scientific skills in structuring an original and productive research program using outstanding communication and collaborative abilities. An individual selected for a tenure-track position is expected to build a dynamic and productive research group. Candidates for a tenured position must have an international reputation and well-documented evidence of ongoing independent accomplishments. Laboratory facilities, start-up and sustained research funds and salary will be competitive with premier academic institutions. Applicants should send curriculum vitae, bibliography, statement of research interests, and names of references to: **Dr. Story Landis, Director, NINDS c/o Peggy Rollins, Office of the Scientific Director, Division of Intramural Research, NINDS, Building 35 Room GA908, NIH, Bethesda, MD 20892, 301-435-2232**. The NINDS is one of the Institutes of the National Institutes of Health, a component of the Department of Health and Human Services. Applications will be reviewed upon receipt.

Health Scientist Administrator

The National Institute on Drug Abuse (NIDA), a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS), is recruiting for a Health Scientist Administrator (HSA). The incumbent serves as HSA in the Science Policy Branch of the Office of Science Policy and Communications. The incumbent: (1) Develops position statements on science and science policy issues related to clinical, behavioral, health services and/or social science research, which requires expert knowledge of how these research areas relate to drug abuse and addiction; (2) responds to inquiries from all levels of the Department, other federal agencies, the scientific community, and the general public; (3) works with program staff to develop, direct, and implement the science-based strategic planning and evaluation processes for NIDA's research programs in the areas of clinical, behavioral, health services or social sciences; and (4) represents the Institute before professional, scientific and public interest groups, as well as interagency task forces.

For additional information on this position, and for instructions on submitting your application, please see the website, at: www.usajobs.com. Detailed information is provided under vacancy announcement number: **NIDA-06-144611** Supplemental documentation must be submitted to: **Nancy Delgais, National Institutes of Health, 111 Alexander Drive, Maildrop NH-01, Research Triangle Park, NC 27709** or faxed to **919-541-3659**.

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Department of Health and Human Services
National Institutes of Health
National Institute of Dental and Craniofacial Research
Health Scientist Administrator

We are seeking outstanding scientists, with doctorate level training, independent research, and administrative experience, to join a team of interactive and diverse group of scientists to help shape the future of state-of-the-art research in oral, dental, and craniofacial biology. The National Institute of Dental and Craniofacial Research (NIDCR), a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services, invites you to apply for the position of Health Scientist Administrator in the Division of Extramural Activities, Scientific Review Branch. The mission of the NIDCR is to improve oral, dental and craniofacial health through research and research training in basic and clinical research on the full spectrum of topics related to oral, dental and craniofacial biology, including oral cancer, chronic pain, immunology, salivary gland physiology, mineralized tissue, craniofacial development and genetics, biomimetics, tissue engineering, and health promotion and behavior.

The incumbent will serve as a Scientific Review Administrator (SRA), and be responsible for the initial administrative and scientific merit review of applications for research programs and/or research training and career development grants through interaction with established scientists in a variety of fields. SRA's are responsible for assuring the fairness and consistency of the scientific peer review process, and for providing technical guidance on peer review policies and procedures and review criteria to applicants, reviewers, and Institute staff.

The salary range for this position is $65,048 to $118,828 per annum, commensurate with qualifications and professional experience. A full benefits package is available, which includes retirement, Thrift Savings Plan participation, health, life and long-term care insurance.

For qualification requirements, evaluation criteria, and application instructions, view the vacancy announcements at **http://jobsearch.usajobs.opm.gov/a9nih.asp**. Refer to announcement **# NIDCR-06-145299DE** and **NIDCR-06-148542MP**. Applications will be accepted until **October 20, 2006**. Please contact **Michelle Lipinski at 301-594-2286 or lipinskim@od.nih.gov** if you have questions.

Center for Scientific Review

Scientific Review Administrator
(Health Scientist Administrator)
Center for Scientific Review
http://cms.csr.nih.gov/

Would you like to work with the most accomplished scientists in your field to provide fair and expert peer review of research and training grant applications submitted to the NIH? The Center for Scientific Review is recruiting dynamic, experienced research scientists in a variety of scientific areas. The successful candidate will be a respected, accomplished scientist with maturity, integrity and outstanding communication skills. Requirements include an M.D. or Ph.D. degree in the biomedical or behavioral sciences (or equivalent training and experience), a record of independent research accomplishments in your field, documented by an outstanding publication record and administrative background.

The Scientific Review Administrator is at the focal point of NIH peer review. SRAs analyze grant applications for key topic areas, recruit experts, conduct study section meetings, and prepare review documents. The position involves travel to scientific meetings, training in health science administration, opportunities to serve the larger NIH community, and career development activities.

Compensation is commensurate with research experience and accomplishments, and a full Civil Service package of benefits is available (including retirement and thrift plans as well as health, life, and long-term care insurance).

For information about current opportunities as a Health Science Administrator at CSR, consult our website: **http://cms.csr.nih.gov/AboutCSR/Employment/**. Feel free to call **(301) 435-1111** as well, if you have any questions.

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Indian Institute of Science Education & Research
Kolkata

Indian Institute of Science Education & Research is set up at Kolkata by the Government of India at par with IITs and IISc Bangalore. The Institute offers a programme of five-year integrated Masters of Science in the Schools of Physical Sciences, Life Sciences and Mathematics & Information Sciences. In addition, there is a full-fledged research activity in a net work arrangement with neighboring institutions. Until the Institute gets its own premises at Haringhata, Kalyani ready, the teaching and research programmes have already started from 16th August 2006 in the Salt lake campuses of IIT and NITTTR. The Institute invites applications from Indian nationals possessing excellent academic record and experiences in teaching and research for the following faculty positions:

1. **Professor**
   Scale of pay : Rs. 18,400-500-22,400/-
2. **Associate Professor**
   Scale of pay : Rs. 16,400-450-20,000/-
3. **Assistant Professor**
   Scale of pay : Rs. 12,000-420-18,300/-

Including allowances as admissible to Central Government Employees in Kolkata.

Qualifications: Ph.D in any area of Mathematics, Biology, Chemistry, Physics, Computer & System Sciences, Earth & Planetary Systems and Engineering Sciences.

Experience: Post doctoral teaching and research experiences, appropriate to the positions applied for.

In addition to pay and allowances the faculty members may be provided with the following:

- Reimbursement of telephone charges up to Rs. 750/- per month
- Rs. 4000/- per year as book grant
- Reimbursement of 75% of membership fee of one international professional society every year
- Financial support to attend national / international Conferences / Seminars / Workshops etc.
- Seed money for starting research laboratory

Accommodation: The campus in Haringhata is going to be fully residential with all amenities. Until the campus is ready, limited accommodation in Kolkata may be available.

Interested candidates may apply enclosing:

- Curriculum Vitae
- List of Publications (with reprints of important papers)
- Names and addresses (with e-mail address and fax number) of at least six referees
- Any other details relevant to the candidature, including a statement of purpose

The qualification & experience prescribed are the minimum and mere possession of the same does not entitle a candidate to be called for interview. However, the experience criteria may be relaxed for exceptionally meritorious candidates.

Application should reach the office of the Director, IISER-Kolkata, IIT Kharagpur Kolkata Campus, HC Block, Sector-III, Saltlake, Kolkata-700 106 on or before **31st October, 2006.**

Director, IISER, Kolkata

Advt. No. IISER-K/2/2006

Max-Planck-Gesellschaft
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Successful applicants will have demonstrated the ability to perform excellent research. They will be offered an

Independent Junior Research Group Leader position

(W2; equivalent to associate professor level without tenure) including a five-year grant (research positions, budget, investments) at one of the following Max Planck Institutes

MPI for Medical Research, Heidelberg
(1 position)
MPI for Experimental Medicine, Göttingen
(1 position)
MPI for Biophysical Chemistry, Göttingen
(2 positions)
MPI for Developmental Biology, Tübingen
(1 position)
MPI of Immunobiology, Freiburg
(2 positions)
MPI for Molecular Plant Physiology, Golm/Potsdam (2 positions)
MPI for Molecular Genetics, Berlin
(1 position)

Applications should include a CV, a list of publications, copies of three publications, a one-page summary of scientific achievements, and a two-page research plan. For further information and detailed application instructions see

http://www.snwg.mpg.de

The Max Planck Society is committed to equal opportunities and to employing disabled persons.

The deadline for application is **October 31, 2006.**

THE NEW LIFE SCIENCES INITIATIVE at Cornell University

CORNELL UNIVERSITY IS CONTINUING AND EXPANDING its $750-million initiative to recruit faculty and provide resources that foster the multidisciplinary study of organisms and to establish Cornell as one of the preeminent comprehensive life science institutions. Cornell's new president, David J. Skorton, MD, and Cornell's provost, Carolyn A. Martin, have recommitted the university to its continuing goal of integrating Cornell's existing great strengths in organismal biology with its outstanding programs in the physical, engineering, and computational sciences to provide fundamental explanations of mechanisms. This goal is facilitated through several interconnected, campus-wide focus areas composing the New Life Sciences Initiative (NLSI). Since 1998 and extending through the next five years, Cornell is making 120 professorial appointments, with 60 new hires to date. This has driven the establishment of several new programs, including the Department of Biological Statistics and Computational Biology; the Department of Biomedical Engineering; the Cornell Center for Vertebrate Genomics; and the Cornell Institute of Cell and Molecular Biology (see column at the right). New research core facilities have also been created and staffed, including Biological Computation; DNA Microarray; DNA Sequencing and Genotyping; Fermentation; Microscopy, Imaging, and Fluorimetry; Mouse Transgenics; Plant Gene Expression; Protein Production and Characterization; and Proteomics and Mass Spectrometry.

The Campus and the Local Area

The main campus of Cornell University, one of the most beautiful in the country, overlooks 40-mile-long Cayuga Lake. It is located in Ithaca in the Finger Lakes region of Upstate New York, a scenic environment of spectacular lakes, waterfalls, gorges, rolling hills, farmland, vineyards, and state parks. It is an area with outstanding recreational and summer and winter sports opportunities for individuals and families. The Ithaca community is culturally diverse with excellent theater, music, sports, and other activities befitting a major university town yet it also has the warmth and friendliness of a small city. The area is known for its many bookstores and restaurants, an extensive walking trail system, arboretum, Laboratory of Ornithology, marina, Farmers' Market, a hands-on Sciencenter, and art and science museums. For more information and links to individual attractions, visit http://www.visitithaca.com/.

As a private endowed university that includes several state-assisted colleges, the state's land-grant institution, and a member of the Ivy League, Cornell composes an unusually varied array of academic units. Cornell's graduate program in biology is ranked seventh in the latest national survey (*US News & World Report*). Biology is Cornell's largest undergraduate major and, according to NSF, sends more of its students on for PhDs in the life sciences than any other university. The NLSI has expanded the graduate fellowship program and has had a marked impact on the curriculum for both graduate and undergraduate students. This includes a new undergraduate major in Computational Biology. New courses have been added—in proteomics and computational biology, for example—while genomics-related topics have been introduced in existing courses in other disciplines across campus.

Schwartz Center for the Performing Arts

The Life Sciences Technology Building and the East Campus Research Facility, both now nearing completion (see images on next pages), together will add 350,000 gross square feet (GSF) to research facilities for life scientists. New York State has recently announced a $50-million contribution to Cornell's new Animal Health Diagnostic Center, which is now in advanced design, and Cornell is spending $100-million to build and renovate campus-wide animal facilities. In addition, a new 196,000-GSF Physical Sciences Building, which will have significant life-science emphasis, is in planning. Many spin-off biotechnology companies are located at the Cornell Business and Technology Park in Ithaca and at the new 72-acre Agriculture and Food Technology Park on Cornell's campus in nearby Geneva, NY.

There is also strong and increasing support for **collaborations between the Ithaca campus and the Weill Cornell Medical College in New York City**, focusing on four areas: Biomedical Engineering, Nanomedicine, and Systems Biology; Cancer Biology; Chemical Biology and Experimental Therapeutics; and Global Health and Infectious Diseases. Cornell's campuses are partners with Rockefeller University and Memorial Sloan-Kettering Cancer Center in the Tri-Institutional Research Program, which cooperatively trains PhD students in Chemical Biology and in Computational Biology and Medicine. A dedicated bus link between the campuses has now been established.

Scott D. Emr

Cornell Institute of Cell and Molecular Biology

Cornell University recently created a new Institute of Cell and Molecular Biology (CICMB) that has as its goal the investigation of fundamental processes essential for the growth and function of all cells. Research in the institute will be focused on the major themes of cell signaling and molecular dynamics. New faculty positions at senior and junior levels, to be advertised in 2007, will be available in the institute starting in early 2008 when the new 265,000-GSF Life Sciences Technology Building is completed (see image on next page). This new research facility will bring scientists in many disciplines including chemistry, biology, physics, engineering, and computer sciences together in one building to work side by side. The building has been designed to maximize interactions and encourage collaboration both among scientists in the building and across the Cornell campus.

Major areas in cell biology including cell cycle control, cytokinesis, receptor signaling networks, cytoskeleton dynamics, organelle biogenesis, regulation of membrane traffic, organelle architecture, and the pathways that coordinate these essential cellular processes will be represented in the institute. New discoveries resulting from work in these areas ultimately will lead to understanding of the

molecular basis for much broader questions in development, neurobiology, immunology, and human disease.

To lead the CICMB, Cornell recently appointed Scott D. Emr, an internationally renowned cell biologist from the University of California at San Diego, as director. Emr currently is Professor of Cellular and Molecular Medicine at the UCSD School of Medicine and an Investigator with the Howard Hughes Medical Institute. He will begin his Cornell appointment in March 2007. Emr and his research team use yeast—a single-celled eukaryotic organism—as a model to understand the complex cellular machinery that sorts and delivers proteins and enzymes to intracellular compartments. Emr's group has identified many new genes that direct these essential protein trafficking pathways, most of which have counterparts in humans. One set of gene products, the ESCRT complexes, play an essential role in turning off signals received by specific growth factor receptors at the cell's surface. The ESCRT complexes have also been shown to play an essential role in the budding and release of the HIV virus from infected cells.

Faculty searches are listed below. Visit our websites: vivo.library.cornell.edu, www.genomics.cornell.edu, and lifesciences.cornell.edu/about/initiative.php. Cornell is an equal opportunity, affirmative action employer. Women and minorities are strongly urged to apply.

Life Science Technology Building (opens January 2008)

Bioengineering and Biomedical Engineering

Life Sciences / Engineering Interface

Cornell seeks a senior professor working at the interface of engineering and life science who thrives on interdisciplinary research and teaching. Cornell has formed a new Department of Biomedical Engineering (BME) that seeks to bridge medicine, biology, and engineering. The department is guided by a vision of developing a quantitative understanding of the human body across scales as a basis for the rational design of devices, diagnostics, and therapies to improve human health. BME is responsible for granting MS/PhD and MEng degrees and sponsors an undergraduate minor in BME available to all students majoring in engineering. We have an opening for a senior tenured faculty member at the level of Associate Professor or Professor with appointment to an endowed chair possible. Candidates must have a truly outstanding record of excellence in research and education. Research in any area of biomedical engineering will be considered, although there are particular needs for faculty with interests in cellular/tissue bioengineering, molecular/cellular imaging technology, biomaterials, systems biology, and applications of micro/nanofabrication in medicine. Cornell's Center for Materials Research, Nanobiotechnology Center, Center for Nanofabrication, Developmental Resource for Biophysical Imaging and Opto-Electronics, and Theory Center provide outstanding facilities to support interdisciplinary research relevant to biomedical engineering. TO APPLY: Interested candidates should send a letter of interest, *curriculum vitae*, brief statement of research and teaching interests, list of recent externally supported research, and names of three references to: Bmep_search-mailbox@cornell.edu Qualifications: Candidates should have a PhD in Biomedical Engineering or related technical field.

Cayuga Lake Marina

Sustainable Energy Systems

Cornell University's College of Engineering has identified sustainable energy systems as a priority area for growth in research and education. We seek an individual for the newly established David Croll Professorship of Sustainable Energy Systems. The David Croll Professor will be expected to lead research and teaching efforts for this strategic priority (www.engineering.cornell.edu/explore/strategic-planning/). We will accept applications from persons with distinguished academic and research backgrounds in any of the engineering and scientific disciplines which contribute to knowledge in the energy field. The individual we are seeking must have an outstanding record of engineering and scientific accomplishment in an area critical to energy systems, and vigorous current activity in an energy system suitable to a sustainable future. The successful candidate must possess a clear and broad vision of the central issues of energy and its environmental impacts; a firm grasp of what universities can contribute; and the ability to develop and provide leadership to a multidisciplinary, university-wide, research and education program devoted to energy and its environmental impacts. The incumbent will have an appointment in one of the departments of the college. Disciplines in the college currently contributing to energy and environmental impacts research are environmental engineering, chemical engineering, mechanical engineering, electrical engineering, materials science and engineering, and earth and atmospheric sciences. The College of Engineering faculty and administration is committed to increasing the diversity of the faculty and creating a supportive climate for under-represented groups and for women. Applications from women and from members of under-represented groups are strongly encouraged. TO APPLY: Send application to: Professor Michael Spencer, Associate Dean of Engineering, 241 Carpenter Hall, Cornell University, Ithaca, NY 14853. Applications may also be sent by email to Engr_ORGSPE@cornell.edu.

Computational Biology

Applications are invited for a tenure-track position in Computational Biology in the Department of Computer Science at Cornell University. Depending on experience, available positions are at the assistant, associate, or full professor level. Applicants must possess a PhD in computer science or PhD in mathematical, biological or physical science with enough expertise in computer science to fit within a CS department. The department requires demonstrated research abilities at the highest level as well as outstanding teaching ability and leadership qualities. Outstanding applicants in all areas of computational biology will be considered. We are especially interested in the area of computational biophysics. To ensure full consideration, applications should be received by January 15, 2007, but will be accepted until all positions are filled. TO APPLY: Submit a *curriculum vitae* and the names of at least three references, with the names of their institutions and their email addresses to Chair, Faculty Recruiting Committee, Department of Computer Science, 4130 Upson Hall, Cornell University, Ithaca NY 14853–7501.

Life Sciences / Physical Sciences Interface

Cornell has undertaken a broad-based initiative to recruit faculty and provide resources that foster multidisciplinary participation in the post-genomics era. Understanding how genes and their protein products generate the complexity and diversity that we know as life is perhaps the greatest scientific challenge of the new millennium. Developments in the physical and engineering sciences will be essential for addressing complex questions in biology and also engineering novel systems based on biological principles. At Cornell we aim to attract exceptionally talented individuals pursuing important research areas in the life sciences with quantitative, multidisciplinary approaches. Key areas of interest include:

(1) **The development and application of physical and chemical tools to study molecu-**

lar events and interactions in living cells. Examples include: the use of imaging methods (e.g., nonlinear laser-scanning microscopy) to monitor the dynamics of molecular ensembles; the application of spectroscopic and fluorescence methods to monitor protein-protein interactions; and the application of chemical synthesis to control macromolecular reactivity.

(2) **New approaches to probing molecular structure and properties.** Examples include the study and manipulation of single molecules by laser tweezers, force microscopy, or electron microscopy; and the application of protein design to understanding macromolecular interactions.

(3) **The generation of advanced materials integrating, mimicking, or expressing biological functionality.** Examples include the development, study, and/or use of new technologies in the areas of microfluidics, polymers, and biomaterials; the design of novel catalysts; and the establishment of new ways to redirect cellular activities by altering enzymatic or transport functions in organisms.

(4) **The development of new computational models and algorithms to better understand biological complexity and enhance experimental observation.** Examples include modeling of the structure and dynamics of gene networks and of signal transduction pathways, system wide analyses of transcription and translation, and the use of computational biology to advance bioinformatics and structural biology.

Appointees will participate in the university-wide, interdisciplinary program in the life sciences (the New Life Sciences Initiative). They will be hired by and thus find their "home" in one of the following departments: Applied and Engineering Physics, Biological and Environmental Engineering, Chemical and Biomolecular Engineering, Chemistry and Chemical Biology, Computer Science, Materials Science and Engineering, Molecular Biology and Genetics, Molecular Medicine, and Physics. TO APPLY: Applicants should send, as a single PDF document, a cover letter stating the potential home department, a *curriculum vitae*, a concise statement of research and teaching interests, copies of relevant publications, and the names of at least three references to: culifesciences@cornell.edu. Review of applications will begin November 1, 2006 and continue until the positions are filled.

Mammalian Genomics

Nutritional Sciences

The Division of Nutritional Sciences at Cornell University is recruiting outstanding scientists for three tenured or tenure-track faculty positions. The successful candidates are expected to develop extramurally funded research programs and contribute to the division's teaching mission. An advanced degree (MD, PhD, DVM, and/or equivalent) is required and postgraduate training highly desirable.

(1) **Functional Mouse Genomics.** The successful candidate (Assistant Professor) is expected to have expertise in genetics, biochemistry, developmental biology, and/or experimental genomics and interest in exploring the interactions among nutrients, metabolism, and the genome in health and disease. Areas of interest include Mammalian Developmental and Metabolic Programming, Epigenetics, and/or Complex Metabolic Diseases.

(2) **Human Metabolism.** The successful candidate (Assistant Professor) is expected to have expertise in human nutrition and an interest in studying the interactions among nutrients, metabolism, and/or genetic variation in human health and disease. Areas of interest include Maternal and Child Nutrition, Obesity, and Genomics/Metabolomics.

(3) **Global Health & Nutrition.** The successful candidate (Jamison Chair, either Associate or Full Professor) is expected to have experience in global public health research and an interest in studying the biological and/or social dimensions of nutrition. Areas of expertise include but are not limited to Social Sciences, Epidemiology, Intervention Targeting and Evaluation, and/or Infectious Disease.

TO APPLY: These faculty searches will be conducted in concert with Cornell University's interdisciplinary initiatives in Life Sciences, Global Health, and/or Social Sciences. Applications will be received at www.nutrition.cornell.edu. Screening of candidates will begin October 5 and will continue until the positions are filled. Salary will be commensurate with the successful candidate's academic credentials and experience. Women and minorities are encouraged to apply.


Ithaca Farmers' Market

Neuroscience

Cornell University has established a campus-wide program in Neuroscience. Individuals using cell biological, molecular, and/or biophysical approaches to biomedical problems in the nervous system will be considered in a search for an assistant professor studying signaling pathways. The position is available in the Department of Molecular Medicine in the New York State College of Veterinary Medicine. Neuroscience faculty will have full access to multiple genomic and life science facilities on campus and are encouraged to form collaborations throughout the campus. TO APPLY: Applicants should submit a *curriculum vitae* and a description of research plans, plus copies of two papers as a single PDF file (max. 5MB) to Gregory Weiland c/o dac20@cornell.edu and arrange for three letters of recommendation to be sent, both electronically (dac20@cornell.edu) and in hard copy, to: Gregory Weiland, Neuroscience Search, Department of Molecular Medicine, Cornell University, Ithaca, NY 14853–6401.


East Campus Research Facility (opens September 2007)

Plant Genomics

Boyce Thompson Institute for Plant Research at Cornell

BTI, an independent not-for-profit research organization, invites applications for up to three tenure-track faculty positions at any level. We seek candidates whose research addresses important questions in biology using plant, plant-microbe, or plant-herbivore systems. Research topics should be complementary to current research at BTI and Cornell. Particular areas of interest include plant metabolism, small molecule biochemistry, enzymology, ecology, and plant interactions with other organisms. Applications from scientists using model systems not currently represented at BTI are encouraged. BTI is located on the central Cornell campus and has a research-oriented environment with state-of-the-art facilities and family-friendly policies. Our location offers superb opportunities for interactions and formal links to appropriate Cornell departments. We encourage applications from women and minorities. TO APPLY: Applicants should submit a *curriculum vitae*, a statement of research interests, and a concise description of research plans (2–3 pages). Please submit applications and have letters from three references sent to Gary Blissard, Search Committee Chair, Boyce Thompson Institute, Tower Road, Ithaca, NY 14853, fax 607–254–1217, e-mail: ee54@cornell.edu. Review of applications will begin on November 1, 2006 and will continue until the positions are filled. Additional information about BTI can be obtained at bti.cornell.edu.

Black-and-White Photographs by Cornell University Photography

DEAN OF THE COLLEGE OF VETERINARY MEDICINE

http://www.cornell.edu/provost/search-vet.cfm

Located in Ithaca, N.Y., Cornell University is a bold, innovative, inclusive and dynamic teaching and research university where staff, faculty, and students alike are challenged to make an enduring contribution to the betterment of humanity.

Cornell University invites applications and nominations for the position of Dean of the College of Veterinary Medicine. The College is recognized internationally as a preeminent veterinary medical institution that is committed to the concept of one biology in advancing the health of animals and people through education, research, and service.

The College is one of twelve academic units on Cornell's Ithaca campus and it partners with the College of Agriculture and Life Sciences, the College of Human Ecology, the College of Engineering, the College of Arts and Sciences, the Faculty of Computing and Information Sciences, and the Weill Cornell Medical College in the university's New Life Sciences Initiative, designed to position Cornell University at the forefront of biomedical and biological research.

The Dean is the chief academic and administrative officer of a College with 125 tenured/tenure-track faculty, 110 clinical/instructional faculty, a staff of 665, and a student body of approximately 330 DVM and 110 MS/PhD students. The Dean oversees the College's programs of education, research, service, and outreach and has responsibility for administering the College and enhancing its relationship with other colleges and the university administration. The Dean is the College's key representative to alumni, commercial partners, donors, organized veterinary medicine, and governmental agencies. As a member of the university's senior administrative team, the Dean reports to the Provost and works with other deans and executive officers on behalf of Cornell as a whole.

The College enjoys a talented and diverse student body, a highly productive faculty, world-class clinical facilities and staff, and dedicated alumni eager to support the College in its mission. The College is home to five academic departments as well as the Cornell University Hospital for Animals, the Animal Health Diagnostic Center, and the Baker Institute for Animal Health.

The ideal candidate will:

- Be a veterinarian with a distinguished record of accomplishment as a scholar;
- Have demonstrated leadership and administrative experience, characterized by being responsive yet decisive, with a record of being a creative and positive force for successful change;
- Be a team builder, able to connect the individual pieces of a large network into a cohesive and synergistic whole;
- Possess excellent communication, problem solving, strategic planning, operational performance improvement, and negotiation skills, combined with the capacity to engage productively with students, faculty, staff, alumni, business leaders and the veterinary professional community to build consensus creatively and persuasively;
- Lead the College at the interface between application and research as the foundation for education, service, and outreach;
- Have a steadfast commitment to excellent veterinary teaching programs and to high-value clinical and diagnostic services;
- Champion interdisciplinary research and education between the College of Veterinary Medicine and other colleges and units throughout the university;
- Be an advocate for veterinary medicine's crucial role in public health, biosecurity, food safety, emerging infectious diseases, and biomedical research;
- Have the necessary familiarity with political process and the drive to keep the College a leader in research, diagnostics, and service;
- Be committed to building and maintaining diversity among the College's administration, faculty, staff, and students; is open to dialog with all groups about their needs and concerns; and
- Be willing and able to foster alumni relations, forge a strong relationship with New York State veterinarians, and cultivate corporate, foundation, and private donors to support the mission of the College through fund raising.

In keeping with Cornell University's commitment to building a culturally diverse administration, faculty, staff and student body, nominations of and applications from underrepresented groups are particularly encouraged. Applications should include a statement of interest and a curriculum vita. Applications and nominations will be kept strictly confidential. The review of materials will begin immediately and continue until the new dean is selected in time to take office in July 2007.

Send materials to: **Veterinary Medicine Dean Search Manager, 440 Day Hall, Cornell University, Ithaca, NY 14853-2801.** Or electronically to: **vetmeddeansearch@cornell.edu**

Cornell University

Cornell University is an Affirmative Action, Equal Opportunity Educator and Employer.

MICHIGAN STATE UNIVERSITY

Walter F. Patenge Endowed Health Sciences Chair
College of Osteopathic Medicine

The Walter F. Patenge Endowed Health Sciences Chair at Michigan State University (MSU) is an endowed professorship in the College of Osteopathic Medicine. This laboratory-based position will be filled by a senior-level molecular geneticist with an outstanding record of funded research in the area of human cancer genetics.

The Patenge Chair is a tenured research-oriented position, with moderate teaching responsibility. The successful applicant will have a superior record of peer-reviewed publication and will possess a research and/or medical degree (e.g., Ph.D., Sc.D., D.O., M.D.). The successful applicant will have a background that demonstrates his/her ability to work synergistically with both scientific and medical colleagues.

The Patenge Chair is being filled as part of a well-funded initiative to increase human genetics research at MSU. MSU offers a highly collegial, interdisciplinary environment with many collaborative opportunities through two MSU community-based medical schools (D.O. and M.D.) and a research-oriented college of nursing—all of which are associated with large health care systems located throughout Michigan.

The Patenge Chair will have access to four genetics diagnostic labs (DNA, cytogenetics, prenatal screening, HLA), a genetic counseling center, strong epidemiology and neuroscience programs, an interdepartmental graduate program in genetics, and strength in research in a wide array of basic sciences. MSU provides excellent research support facilities in genomics, proteomics, and microscopy.

The successful candidate will be appointed in one or more of the following departments: Microbiology and Molecular Genetics, Biochemistry & Molecular Biology, Physiology, and Pharmacology and Toxicology. Candidates with a professional degree will be jointly appointed in an appropriate clinical department.

The position entails a tenure stream, or tenured associate or full professorship in the department(s) appropriate to the candidate's research interests and experience, a salary commensurate with an endowed professorship, a suitable setup package, and annual interest from the endowment for furthering the candidate's research.

Applicants should submit a letter of application, curriculum vitae, statement of research goals, copies of pertinent reprints and contact information (address, e-mail, and phone) for three referees to: **J. Justin McCormick, Ph.D., Chairperson, Patenge Chair Search Committee, A314 East Fee Hall, East Lansing, MI 48824-1316. 517/432-2821; e-mail: patenge.search@hc.msu.edu**.

Digital submissions in PDF format are encouraged. Review of applications will begin on October 20, 2006.

The position is open immediately and will remain posted until filled. Persons with disabilities have the right to request and receive reasonable accommodation.

MSU IS AN AFFIRMATIVE ACTION, EQUAL OPPORTUNITY INSTITUTION.

ASSISTANT/ASSOCIATE PROFESSOR/PROFESSOR GROSS ANATOMY MEDICAL COLLEGE OF GEORGIA

Department of Cellular Biology and Anatomy at the **Medical College of Georgia**, Augusta, GA, invites applications for a full-time or part-time faculty position at the rank of Assistant/Associate, or full Professor. The successful candidate will take part in teaching cadaver-based human anatomy to medical students and allied health students. Candidates must have a Ph.D. or M.D. and have a minimum of 2 years of teaching experience in Gross Anatomy. Salary is dependent on qualifications and experience. The Medical College of Georgia is a state supported comprehensive medical school whose mission is to train physicians and other health professionals to meet the health care needs of the state. The Department of Cellular Biology and Anatomy prides itself in state of the art interdisciplinary research and excellence in education. Applicants should submit a letter with description of teaching interests and experience, curriculum vitae, and names of three references to: **Adarsh Gulati, Ph.D., Chair, search committee, Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA 30912-2000; Email: agulati@mail.mcg.edu**. Review of applications will begin immediately and continue until the position is filled. PO# E-0791175.

The Medical College of Georgia is an Equal Employment Opportunity and Equal Access Institution.

TENURE-TRACK FACULTY POSITION

MEDICAL COLLEGE OF WISCONSIN

DEPARTMENT OF BIOCHEMISTRY

The Department of Biochemistry is seeking candidates for an Assistant Professor that uses **structural biological approaches** (preference given to X-ray crystallography) to study important biomedical problems. Applicants must have a doctoral degree (Ph.D., M.D., or both), at least 2 years of postdoctoral training, a strong publication record, and potential to obtain outside funding. Competitive salary support, start-up funds and laboratory space in a new research building will be provided.

The Medical College of Wisconsin (**www.mcw.edu**) is the largest private research institution in Wisconsin, conducting $119 million annually in funded research, and over the past few years has been among the fastest growing research-oriented medical schools in the United States for NIH funding. In addition to a strong core of basic biomedical science departments, the Medical College is home to nine internationally and federally designated Centers of Biomedical Research. Excellent shared facilities are available for proteomics, imaging, molecular biology, and mouse genetics and the Department of Biochemistry is home to state-of-the-art X-ray and NMR facilities. The College is conveniently located in suburban Milwaukee and is 8 miles west of Lake Michigan with easy access to surrounding communities, lakes, and parks. For more information about the Department of Biochemistry visit our website at **www.mcw.edu/biochemistry**.

Applications should include a cover letter, curriculum vitae, statement of research interests, and three reference letters. Review of applicants will begin on **October 15, 2006**. For full consideration applications should be received by **November 30, 2006**. Send application materials and reference letters preferably by e-mail, with attached pdf, to **cricker@mcw.edu** or by mail to: **Dr. Robert Deschenes, Chairman, Department of Biochemistry, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226.**

EOE M/F/D/V
www.mcw.edu/hr

MICHIGAN STATE UNIVERSITY

Two Faculty Positions in Human Genetics

Two faculty positions in human genetics are available at Michigan State University (MSU) as part of a campus-wide initiative to increase human genetics research on campus. Individuals are sought for one junior level (assistant professor) and one senior level position.

Research will address fundamental questions of human genetics. At least one position is intended for a candidate having research experience in statistical genetics/genetic epidemiology with an emphasis on complex diseases. In addition, a laboratory-based human geneticist is sought.

MSU offers a highly collegial, interdisciplinary environment with many collaborative opportunities through two MSU community-based medical schools (MD and DO) associated with large health care systems in six cities, four genetics diagnostic labs (DNA, cytogenetics, prenatal screening, HLA), a genetic counseling center, strong epidemiology, cell & molecular biology and neuroscience programs, an interdepartmental graduate program in genetics, and strength in research in a wide array of basic sciences. Michigan State provides excellent research core facilities in genomics, proteomics, and microscopy.

Each successful candidate may choose an academic affiliation among several departments, including Microbiology & Molecular Genetics, Clinical genetics section of Pediatrics & Human Development, Epidemiology, Biochemistry & Molecular Biology, Pathobiology & Diagnostic Investigation, Physiology, Psychology and Pharmacology & Toxicology.

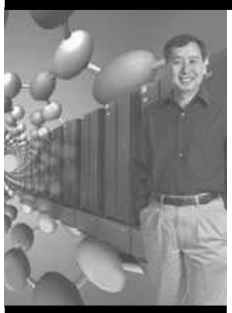
These are academic-year, tenure-track, research-oriented positions, with moderate teaching responsibility. A doctoral degree (PhD, MD, DVM, or DO) and research experience are required. Salary will be commensurate with experience. Applicants should submit a letter of application, curriculum vitae, statement of research goals, copies of pertinent reprints and contact information (address, e-mail, and phone) for three referees to the following address: **Karen Friderici, Professor, Human Genetics Search Committee Chairperson, Genetics Program, Biomedical & Physical Sciences Building, Rm 2240, Michigan State University, East Lansing, MI 48824**.

Digital submissions in pdf format to **genetics@msu.edu** are encouraged. Review of applications will begin on October 20, 2006 and will continue until the position is filled.

Women and minorities are strongly encouraged to apply. Persons with disabilities have the right to request and receive reasonable accommodation.

MSU IS AN AFFIRMATIVE ACTION, EQUAL OPPORTUNITY INSTITUTION.

Physicist Scientist/Engineer or Staff Scientist/Engineer



Lawrence Berkeley National Laboratory (LBNL) is located in the San Francisco Bay Area on a 200-acre site in the hills above the University of California's Berkeley campus and is managed by the University. A leader in science and engineering research for more than 75 years, LBNL is the oldest of the U.S. Department of Energy's National Laboratories.

Reporting to the Division Deputy for the Scientific Support Group (SSG) of the Advanced Light Source (ALS) Division, the incumbent will function as an experimental physicist, supporting and planning experiments to meet the Molecular Environmental Science (MES) and SSG's programmatic plans and R&D goals. The incumbent will be responsible for the operation, maintenance, and upgrades of ALS undulator soft x-ray beamline 11.0.2 and its associated endstations. To learn more about the ALS please visit our web site at **http://www-als.lbl.gov**.

Duties: The candidate will join a research program as applied to environmental science and material science on an undulator soft x-ray beamline (11.0.2) within the SSG of the ALS. The successful applicant will develop the techniques and use of synchrotron radiation using scanning transmission x-ray microscope (STXM), photoemission spectroscopy, x-ray absorption spectroscopy, and ambient pressure spectroscopy for the study of electronic and atomic structure of materials. The appointee will contribute to the experimental program using any or all of the aforementioned techniques with the goal of developing an active community of ALS users of high-resolution spectromicroscopy. The appointee, working closely with the ALS users will design novel experiments and sample preparation techniques to facilitate new results in these fields, and assist in data analysis, interpretation, and simulation of results.

Qualifications: A Ph.D. in Chemistry or Physics, or equivalent experience in a related field is required. A substantial published record of work in materials science and related fields using synchrotron radiation is essential, as well as detailed knowledge of sample preparation, experimental setups, and data analysis for carrying out experiments in the field of high spatial and time resolution spectromicroscopy, photoemission, NEXAFS, and x-ray fluorescence with synchrotron radiation. Familiarity with the design and operation of an undulator based soft x-ray beamline is required, in addition to knowledge of precise motion control and C++ programming. Demonstrated experience in the design, commissioning, and operation of high-resolution beamlines at synchrotron facilities would be beneficial.

NOTE: This career position will be hired at either the scientist or staff scientist level, depending on qualifications and experience.

For fastest consideration, apply online at **http://jobs.lbl.gov**, select "Search Jobs", and enter **019401** in the keyword search field. Enter "Science" as your source.

LBNL is an Affirmative Action/Equal Opportunity Employer committed to the development of a diverse workforce.

For more information about LBNL and its programs, visit **www.lbl.gov**.

UNMC EPPLEY Cancer Center

University of Nebraska Medical Center

Associate Director for Basic Research

The University of Nebraska Medical Center (UNMC) Eppley Cancer Center, a National Cancer Institute-designated Cancer Center, seeks outstanding candidates for the position of Associate Director for Basic Research. This Associate Director position will include a tenured appointment with academic rank commensurate with experience.

The successful applicant will be responsible for the overall direction and development of the Cancer Center's basic research programs. Responsibilities include maintaining an independent research program and fostering the continued development of basic research programs and interdisciplinary collaborations. This person will advise the Director on promising areas of research, provide direction to faculty members in pursuing research objectives, and be responsible for the Cancer Center's basic research shared facilities.

The UNMC Eppley Cancer Center is in a dynamic growth phase and committed to expansion of all its research programs. Growth in the cancer research programs is aided by generous support from the Nebraska Tobacco Settlement Biomedical Research Funds. With a strong commitment of both public and private funds, UNMC has made strategic investments in its research infrastructure with the addition of the Durham Research Center and the Lied Transplant Center, which provide state-of-the-art laboratory and clinical space for cancer research. UNMC is currently building another 240,000 square foot research building, which will provide additional space for continued growth of the Cancer Center.

Applicants should have a history of significant peer-reviewed funding, strong interpersonal and communication skills, and evidence of successful scientific collaborations. Experience in a leadership position within an NCI-designated Cancer Center is preferred. The position includes a generous start-up package and a primary appointment in the Eppley Institute for Research in Cancer and Allied Diseases.

Candidates should have a Ph.D. and/or M.D. degree. Applicants must apply online to position # 1015 at **https://jobs.unmc.edu**. Additional information can be found at **http://www.unmc.edu/cancercenter/**. Candidates should forward a minimum of 3 letters of reference to: **Kenneth H. Cowan, M.D., Ph.D., Director, Eppley Institute for Research in Cancer, Director, UNMC Eppley Cancer Center, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198-6805; kcowan@unmc.edu.**

The University of Nebraska Medical Center is an Equal Opportunity Employer.

THE UNIVERSITY OF HONG KONG

香 港 大 學

The University of Hong Kong is at the international forefront of higher learning and research, with more than 100 teaching departments and sub-divisions of studies, and more than 60 research institutes and centres. It has over 20,000 undergraduate and postgraduate students from 48 countries. English is the medium of instruction. The University is committed to international standards for excellence in scholarship and research.

Post-Doctoral Fellowships

Applications are invited for a number of positions as Post-Doctoral Fellow (PDF) (Ref: RF-2006/2007-128) at the University of Hong Kong, tenable on or before July 31, 2007. Appointments will be made for a period of 2-3 years.

PDF posts are created specifically to bring new impetus and vigour to the University's research enterprise. Positions are available from time to time to meet the strategic research needs identified by the University. In this round of advertisement, PDF positions are available in the following Faculties/Departments/School/Centre/Institute:

- Architecture
- School of Humanities (Philosophy)
- Real Estate and Construction
- Faculty of Education
- Faculty of Dentistry
- Centre of Cancer Research
- Computer Science
- Microbiology
- Medicine
- Chemistry
- Surgery
- Physics
- Ecology & Biodiversity
- Institute of Molecular Technology for Drug Discovery and Synthesis
- Psychology

Requirements

PDFs are expected to devote full-time to research. Applicants should be doctoral degree holders having undertaken original research that has contributed to the body of knowledge. A highly competitive salary commensurate with qualifications and experience will be offered. Annual leave and medical benefits will also be available.

Procedures

Prospective applicants are invited to visit the following webpage <https://extranet.hku.hk/apptunit/> to view the full list of the research areas and their home Faculties/Departments/Schools/Centres for which PDF positions are currently available. Before preparing an application they should contact the Head of the appropriate academic unit to ascertain that their research expertise matches the research area for which a vacant PDF post is available.

Applicants must submit a completed University application form, which should clearly state **which position** they are applying for; and in which **academic discipline**. They should also provide further information such as details of their research experience, publications, research proposals, etc.

Further particulars and application forms (272/302 amended) can be obtained at https://extranet.hku.hk/apptunit/; or from the Appointments Unit (Senior), Registry, The University of Hong Kong, Hong Kong (Fax (852) 2540 6735 or 2559 2058; E-mail: apptunit@hkucc.hku.hk). **Closes October 9, 2006**. Candidates who are not contacted within 3 months of the closing date may consider their applications unsuccessful.

The University is an equal opportunity employer and is committed to a No-Smoking Policy

Nebraska Center for VIROLOGY

Faculty positions to conduct research in a state-of-the-art new research facility through the Nebraska Center for Virology/School of Biological Sciences where excellent opportunities exist for collaboration within the Center and with other departments and institutions.

Associate/Full Professor in Viral Immunology - This is the first of two tenure-line immunology positions to be filled. Candidates should hold a PhD or equivalent, have a strong research record in studying the immune response against viral infection, and have an externally funded research program. Will be expected to further expand an active, extramurally funded research program, show strong leadership in developing a vibrant viral immunology program, and be involved in graduate and undergraduate teaching.

Research Assistant Professor in molecular virology to participate in established research program of the Center's director, **Dr. Charles Wood**, on HIV and AIDS-associated diseases including Kaposi's Sarcoma. Candidates should hold PhD or equivalent with relevant research experience. See our website at: **http://www.unl.edu/virologycenter**

For more information or to apply for this position, visit:
http://employment.unl.edu

To be considered you MUST complete the Faculty/Administrative Information form for requisition number **060786** or **060785** and attach required documents. Review begins **October 31, 2006**.

The University of Nebraska is committed to a pluralistic campus community through affirmative action and equal opportunity. We assure reasonable accommodation under the Americans with Disabilities Act; contact J. Walker at (402) 472-4560 for assistance.

University of Nebraska Lincoln

BCM
Baylor College of Medicine

Tenure Track Faculty Position in Cognitive/ Computational Neuroscience

The Department of Neuroscience at Baylor College of Medicine is undergoing a major expansion of its programs in cognitive and computational neuroscience. Three faculty members will be hired over the next three years to build on existing strengths of the six current faculty in these areas of neuroscience. Candidates should have the Ph.D. degree and postdoctoral training in a field such as Neuroscience, Theoretical Computer Science or Applied Math, or in a quantitative Social Science with a research focus in some area of cognition such as decision-making, social interaction or group dynamics with an emphasis on computational and/or mathematical approaches. The successful candidate may have strong skills and interest in the application of human functional brain imaging, behavioral genetics and/or computational approaches for the analysis of cognition. Major available facilities include two (soon to be increased to three) 3.0 Tesla research-dedicated fMRIs for human imaging, behavioral observation and testing facilities, extensive data collection, management and analysis hardware and software systems, including a unique hyperscanning facility for simultaneous fMRI studies in socially interacting groups as well as human gene sequencing facilities and experimental electrophysiology laboratories.

Send *curriculum vitae* and statement of research interests electronically to **friedlan@bcm.edu** and have at least three letters of reference sent to: **Michael J. Friedlander, Ph.D., Chair, Department of Neuroscience and Director of Neuroscience Initiatives, Baylor College of Medicine, One Baylor Plaza, Suite S740A, Houston, TX, 77030** by **November 15, 2006**. Visit our departmental website at: **http://neuro.neusc.bcm.tmc.edu** for more information.

Baylor College of Medicine is an Equal Opportunity/ Affirmative Action and Equal Access Employer.

USC

Assistant/Associate Professor Medicinal Chemistry School of Pharmacy

The University of Southern California Department of Pharmacology and Pharmaceutical Sciences (**http://www.usc.edu/schools/pharmacy/departments**) invites applications for an Assistant/Associate Professor position, tenure-track or tenured, to expand its faculty in medicinal chemistry. The successful candidate should have a doctoral degree and postdoctoral experience in medicinal chemistry, organic chemistry or related disciplines. The successful candidate is expected to develop a strong research program with extramural funding that complements and expands existing departmental strengths in drug design and discovery, epithelial cell biology, membrane trafficking, drug delivery, pharmacokinetic imaging, neurobiology and aging. Candidates with research interest in neurochemistry or developmental therapeutics for cancer or acquired genetic diseases and an ability to work at the biological interface are particularly encouraged to apply.

The University of Southern California offers cutting-edge opportunities for multidisciplinary, interdisciplinary and translational research collaborations, including an NCI-designated Comprehensive Cancer Center, the USC Provost's Initiatives (Biomedical Imaging Science, Biomedical Nanoscience, Neuroscience and others), iPIDD, a joint interdisciplinary program in drug discovery with the Department of Chemistry of the College, a Center for Stem Cell and Regenerative Medicine, etc. Furthermore, the University offers access to one of the widest variety of affiliated private and public hospitals in the United States (**http://www.usc.edu/health/ClinHospPharm.html**).

Candidates should send the names of three references, a curriculum vitae, and a summary of research accomplishments and future research and educational goals to: **Walter Wolf, PhD, Chair, Medicinal Chemistry Search Committee, University of Southern California School of Pharmacy, 1985 Zonal Avenue, Los Angeles CA 90089-9121** or email **wwolfw@usc.edu**. Review of applications will begin immediately, and will continue until the position is filled.

The University of Southern California is an Equal Opportunity/Affirmative Action Employer and encourages applications from women and minorities, and provides reasonable accommodation to individuals with known disabilities.

Health Equity Initiatives, Professor of Biology San Francisco State University

We are searching for an Associate or Full Professor that will lead Health Equity Initiative (HEI) activities in the College of Science and Engineering (CoSE). Working with HEI Director, **Dr. Cynthia Gomez**, the HEI Professor of Biology will collaborate with HEI Professors in Sociology and Health Education to enhance the capacity of the SFSU community to obtain funding for interdisciplinary research, community interventions, curricular offerings and training programs that address health disparities. Qualifications are a Ph.D. degree, postdoctoral training, a proven research record, evidence of significant grant funding, as well as strong teaching and mentoring experience. The successful candidate will have: (1) an active Biology research program focused on an area integral to the study of health disparities; (2) a substantial record of extramural funding; and (3) demonstrated ability to lead and mentor faculty in the development of collaborative grant proposals. Furthermore, the HEI Professor of Biology is expected to increase student awareness of, and interest in, the promotion of health equity through service and professional development. Therefore the successful candidate will teach one course per semester, and coordinate on-going efforts in CoSE to develop educational modules based on health disparity research.

San Francisco State University, a member of the California State University system, serves a diverse student body of 29,000 undergraduate and graduate students. The mission of the University is to promote scholarship, freedom, human diversity, excellence in instruction, and intellectual accomplishment. San Francisco State University faculty are expected to be effective teachers and to demonstrate professional achievement and growth through continued research, publications, and/or creative activities.

Applicants should send a cover letter, curriculum vitae, and the names and contact information for three references to: **HEI Professor of Biology Search, Department of Biology, San Francisco State University, 1600 Holloway Ave, San Francisco, CA 94132**. Review of applications begins **October 15, 2006** and the expected start date is August, 2007. For additional information about the Department of Biology and five open tenure-track positions, please visit our web site at **http://www.sfsu.edu/~biology**.

SFSU is an Affirmative Action/Equal Opportunity Employer.

ENDOCYTE
PhD and PostDoctoral Fellowships

ENDOCYTE is a multidisciplinary Research and Training Network (RTN) funded by the 6th Framework Programme of the European Union focused on the relevance of the intracellular routes of growth factor signalling for the regulation and diversification of growth factor function.

This Consortium is seeking enthusiastic and highly motivated PhD students and PostDoctoral fellows, who will enrol in one of 12 Research Projects at a partner laboratory:

C. Ibáñez (coordinator)	Stockholm (Sweden)
M. Fainzilber	Rehovot (Israel)
I. Dikic	Frankfurt (Germany)
P. Bastiaens	Dortmund (Germany)
H. Waldmann	Dortmund (Germany)
I. Guerrero	Madrid (Spain)
G. Schiavo	London (UK)
H. Stenmark	Oslo (Norway)
G. van der Goot	Lausanne (Switzerland)
E. Fisher	London (UK)
L. Buday	Budapest (Hungary)
R. Eils	Heidelberg (Germany)

For a full description of the projects and further details of the Consortium aims and training, please access the ENDOCYTE web resource at **http://www.endocyte.ki.se**. Applications should be addressed to **mike.fainzilber@weizmann.ac.il** (PostDoctoral fellows) or to **gisou.vandergoot@epfl.ch** (PhD students) indicating three project preferences. Applications will be considered on an ongoing basis until all positions are filled.

BCM
Baylor College of Medicine

Faculty Position in Systems' Neuroscience Department of Neuroscience

As part of a major initiative in neuroscience, Baylor College of Medicine is recruiting a tenure track faculty member in systems' neuroscience who is interested in the study of sensory, motor or integrative processes including cognition under normal conditions or in disease. The successful candidate for this position will have the Ph.D. and/or M.D. degree and postdoctoral training that includes a record of accomplishment in systems' neuroscience. Successful candidates will have existing or demonstrated potential for extramural research grant support. Candidates who utilize research tools that may include imaging, electrophysiology in alert preparations and/or innovative behavioral paradigms with animal models ranging from primates to invertebrates are encouraged to apply.

Send *curriculum vitae* and statement of research interests/plans electronically to **friedlan@bcm.edu** and have at least three letters of reference sent to: **Michael J. Friedlander, Chair, Department of Neuroscience and Director of Neuroscience Initiatives, Baylor College of Medicine, One Baylor Plaza, Houston, Texas, 77030** by **November 15, 2006**. Visit our department website at: **http://neuro.neusc.bcm.tmc.edu/**.

Baylor College of Medicine is an Equal Opportunity/ Affirmative Action and Equal Access Employer.

National University of Ireland, Galway
Ollscoil na hÉireann, Gaillimh

PROFESSORSHIP OF MOLECULAR MEDICINE

The National University of Ireland, Galway seeks applications from outstanding individuals for the newly established position of Professorship of Molecular Medicine. The University has recognised strengths in biomedical research, and is committed to significant strategic recruitment in this area. The University's commitment to biomedical research is reflected by the establishment of the National Centre for Biomedical Engineering Science (NCBES) and the associated Regenerative Medicine Institute (REMEDI) on campus. Priority biomedical research areas include programmes in cardiovascular disease, orthopaedics, neuroscience and cancer.

Supported by generous philanthropic funding, the Chair will provide leadership, vision and direction in the broad area of Molecular Medicine, and will be expected to contribute to the translational goals of the NCBES. While we encourage applications from outstanding individuals with a proven track record of achievement in any of our priority research areas, arising from our commitment to the development of a Comprehensive Cancer Centre in partnership with University College Hospital, Galway, we are particularly interested in applications from individuals with a demonstrable track record of achievement and productivity in an area of cancer research that is synergistic with our core strengths.

Minimum requirements include a PhD, MD, or an MD/PhD with significant research experience, and a track record of sustained accomplishments and evidence of leadership in his/her field.

For informal discussion, please contact Prof. Terry Smith, Professor of Biomedical Engineering Science and Director, National Centre for Biomedical Engineering Science (NCBES), NUI Galway. Tel.: +353-91-512307; Fax: +353-91-750596; E-mail: terry.smith@nuigalway.ie. For additional information on NUI Galway's biomedical research programmes, candidates are encouraged to visit our website at http://www.ncbes.ie and http://www.remedi.ie .

Salary: €106,914 x 7 = €136,298

Closing date for receipt of applications is Friday November 24th 2006.

Further information on the above post is available from the Human Resources website: http://www.nuigalway.ie/vacancies

Human Resources Office,
NUI Galway, Galway.

Tel. 353 91 492151;
Email: hr@nuigalway.ie;

National University of Ireland, Galway is an equal opportunities employer.

www.nuigalway.ie

THE UNIVERSITY of NORTH CAROLINA GREENSBORO

Molecular Neurobiologist – Faculty Position

The Department of Biology invites applications for an OPEN RANK, full-time, tenure-track position in **Molecular Neurobiology**. A Ph.D. and postdoctoral experience is required for candidates at the rank of Assistant Professor; commensurate expectations are held for candidates seeking appointment at the Professor or Associate Professor rank. Candidates are expected to have an outstanding record of research achievement and to attract extramural funding for a research program that involves undergraduate and graduate students. Candidates at the rank of Associate Professor or Professor must have a solid record of external funding. We welcome applications from scientists with research in any area of molecular neurosciences, including developmental neurobiology, neurogenetics, nanobiosciences, neurobiology of aging, and the role that environmental influences have on molecular aspects of neurophysiology and neurobiology.

The Department of Biology at UNCG includes 21 tenure-stream faculty. Departmental facilities include confocal microscopy, scanning electron microscopy, automated sequencers, gene array analysis, animal facilities, and core biotechnology and tissue culture labs. The department has active research programs in signal transduction, genetics/genomics, developmental biology, cell biology, freshwater/riparian ecology, environmental forensics, and includes a number of researchers using model systems such as Drosophila, Arabidopsis, Xenopus, and mice. Greensboro, located in central North Carolina, provides a high standard of living and is an excellent location for interactions with North Carolina's vibrant biomedical/biotechnological communities at nearby universities and medical schools.

Applicant should submit **(1) a cover letter, (2) a curriculum vitae, (3) a statement of research interests, (4) a statement of teaching experiences/interests, and (5) three letters of reference**, to be sent to:

Dr. Dennis LaJeunesse, Chair of the Molecular Neurobiology Search Committee, The University of North Carolina at Greensboro, Department of Biology, 312 Eberhart Bldg., Greensboro, NC, 27402-6170

Review of applications begins on December 11th, 2006, and will continue until the position is filled. For more information, visit our website at http://www.uncg.edu/bio

EEO/AA

CASE

CASE WESTERN RESERVE UNIVERSITY
SCHOOL OF MEDICINE

Faculty Positions
Department of Pharmacology

Applications are invited from dynamic scientists for several faculty positions in the growing Department of Pharmacology at the Case Western Reserve University School of Medicine. Faculty rank from Assistant to Full Professor is open, dependent on current level of achievement.

The Department has a great tradition of excellence in molecular pharmacology with strong, growing programs in cell regulation and signaling, membrane structural biology, cancer cell biology, and an evolving emphasis on translational pharmacology. The goal of the search is to add to existing strengths in the Department and/or the School of Medicine. The best candidates in any area relevant to modern pharmacology will be competitive. Visit our website **http://pharmacology.case.edu/**.

Applicants should submit a cover letter, their full Curriculum Vitae with publications and grant support, and a list of professional references. In addition, all applications should include descriptions of the applicant's research interests and goals, and teaching, mentoring, and professional service experiences.

Applications should be transmitted by email to **Amy Wilson-Delfosse, Ph.D.,** Associate Professor of Pharmacology (**axw41@case.edu**), with C.C. to **Camala Thompson**, Programs Administrator (**cami@case.edu**).

In employment, as in education, Case Western Reserve University is committed to Equal Opportunity and World Class Diversity.

Assistant/Associate or Full Professor
Department of Cellular Biology and Anatomy
Medical College of Georgia

The Department of Cellular Biology and Anatomy at the **Medical College of Georgia** is seeking tenure track faculty at the Assistant/Associate or Full Professor level. The area of research is open, but should complement existing strengths in vision, musculoskeletal biology, wound healing, and tissue regeneration. Excellent opportunities exist for collaboration using state-of-the-art core facilities in imaging, transgenic mouse and zebrafish, proteomics, electron microscopy, flow cytometry, and molecular biology. Candidates must have a Ph.D. and/or M.D., postdoctoral experience, a strong publication record, and potential for extramural funding at the Assistant and significant current funding at the Associate/Full Professor level. The successful candidate will receive a competitive start-up package, be expected to establish and maintain an extramurally funded research program, and participate in teaching. Applicants should send a curriculum vitae, statement of research objectives, description of teaching interests, and the names of three references to: **Dr. Mark W. Hamrick, Chair, Search Committee, Department of Cellular Biology & Anatomy, Medical College of Georgia, Augusta, GA 30912-2000; Email: mhamrick@mail.mcg.edu**. The Medical College of Georgia is the health sciences campus of the University System of Georgia. It is located in the historic city of Augusta, Georgia with excellent recreational and lifestyle opportunities. Review of applications will begin immediately and continue until the position is filled. PO# E-0791171. *The Medical College of Georgia is an Equal Employment Opportunity and Equal Access Institution.*

ECOLOGICAL/EVOLUTIONARY BIOLOGY

As part of an ongoing expansion of the Biological Sciences faculty, **Kent State University** invites applications for a tenure-track position in the Department of Biological Sciences at the rank of Assistant Professor beginning in August 2007. We seek applicants who complement departmental strengths in conservation biology, ecology, and evolutionary biology and are particularly interested in candidates using genomic and/or proteomic tools to address ecological and/or evolutionary questions. The department has access to a variety of field sites and superb core research facilities and offers competitive startup packages. The successful candidate is expected to establish a high-quality, extramurally funded research program and exhibit a commitment to excellence in graduate and undergraduate education. Applicants must have a Ph.D. degree and postdoctoral experience. For more information on this position and the faculty, see **www.kent.edu/biology/ecol_search.cfm**.

Applicants should send their curriculum vitae and relevant reprints, statement of research and teaching interests, and three letters of recommendation to: **Chair, Ecological/Evolutionary Biology Search Committee, Department of Biological Sciences, Kent State University, P.O. Box 5190, Kent, Ohio 44242-0001; Fax: 330-672-3713**. Review of applications will begin **October 31, 2006**, and continue until the position is filled.

Kent State University is an Affirmative Action/Equal Opportunity Employer and encourages applications from candidates who would enhance the diversity of the University's faculty.

UCI
University of California, Irvine

Assistant or Associate Professor, Developmental Biology

The Department of Developmental and Cell Biology invites applications for a faculty appointment in the area of developmental biology, with an emphasis on organogenesis. We are particularly seeking candidates that take genetic approaches in vertebrate model systems to study formation of complex organs and/or tissue patterning. The successful applicant is expected to conduct a strong research program and to contribute to the teaching of undergraduate and graduate students.

Contact information: Please see the URL: **http://jobs.bio.uci.edu/showopenjobs_tenure.cfm** for application instructions under "Department of Developmental and Cell Biology."

DEADLINE FOR RECEIPT OF APPLICATIONS: Review of applications will begin **December 10, 2006** and the recruitment will remain open until a suitable candidate has been hired.

The University of California, Irvine is an Equal Opportunity Employer committed to excellence through diversity and strongly encourages applications from all qualified applicants including women and minorities.

USF HEALTH UNIVERSITY OF SOUTH FLORIDA

http://hsc.usf.edu · 12901 Bruce B. Downs Blvd, MDC 02 · Tampa, FL 33612

ASSISTANT/ASSOCIATE PROFESSOR

Department of Molecular Pharmacology & Physiology
School of Basic Biomedical Sciences
University of South Florida Heath, College of Medicine

The Department of Molecular Pharmacology & Physiology of the School of Basic Biomedical Sciences within the College of Medicine at USF Health is accepting applications for two tenure/tenure earning pathway faculty positions at the level of Assistant/Associate Professor in molecular and cellular cardiology, vascular biology or cardiovascular pharmacology.

USF Health has at its core the colleges of Medicine, Nursing, and Public Health. Also included are the schools of Basic Biomedical Sciences and Physical Therapy. Currently, the College of Medicine has over 540 fulltime core faculty, over 450 medical students, and over 150 students in the biomedical graduate program. In partnership with its affiliated hospitals, USF Health's research funding last year was $134 million - more than half of which came from federal sources. It has research and clinical affiliations with Pepin Heart Hospital & Research Institute, H. Lee Moffitt Cancer Center, the Johnnie B. Byrd Sr. Alzheimer's Research Institute, the Tampa General Hospital, the All Children's Hospital, the Shriner's Hospital for Children, and the James A. Haley and Bay Pines VA Medical Centers. USF is one of only 95 public and private universities in the U.S. that have been designated as Carnegie Comprehensive Doctoral Research University/Very High Research Activity.

Minimum requirements for Assistant Professor include a MD, PhD, or MD/PhD with a minimum of two years of experience as a junior faculty and evidence of continued growth as an educator, mentor, and a productive researcher. For Associate Professor, a minimum of five years of continuous and productive accomplishment as an Assistant Professor at a university, or an equivalent research institution is required. The successful candidate is expected to have a distinguished record of scholarly activity, NIH R01 and other extramural funding and teaching experience in a medical/graduate curriculum. A legacy of building interdisciplinary programs and experience with successfully mentoring graduate and medical students and postdoctoral-fellows is desired.

Applicants should submit, by email, a letter summarizing their qualifications and interests in the position, future research plans, updated curriculum vitae and the names and contact information of five professional references. Completed applications must be submitted to **Ms. Vanessa Ayer (vayer@health.usf.edu)**. All inquiries and applications will be treated confidentially. Competitive start-up packages and salaries will be provided commensurate with experience. Review of applications will begin October 1, 2006. EOE

50 YEARS CELEBRATING SUCCESS

UNC SCHOOL OF MEDICINE
DEPARTMENT OF PHARMACOLOGY

Tenure-track Faculty Position

The Department of Pharmacology at the University of North Carolina at Chapel Hill invites applications for a tenure-track faculty position. We seek candidates with research interests in molecular and structural aspects of signal transduction and human disease. Candidates with expertise in structural biology, chemical genetics, model organisms, or drug discovery are especially encouraged to apply.

The successful candidate will be expected to direct an independent research program supported by extramural funding, participate in graduate training, and teach medical and dental students. An excellent start-up package and access to departmental and institutional facilities will be provided. Current research strengths of the Department can be viewed at **http://www.med.unc.edu/pharm/**.

Candidates should submit a curriculum vitae, a statement of current and future research plans, selected recent publications and three letters of reference to:

Faculty Search Committee
Attn: Arlene C. Sandoval
Department of Pharmacology, CB #7365
1108 Mary Ellen Jones Building
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27599-7365

Application deadline: Open until filled

The University of North Carolina at Chapel Hill is an Equal Opportunity/ADA Employer.

USF HEALTH UNIVERSITY OF SOUTH FLORIDA

http://hsc.usf.edu · 12901 Bruce B. Downs Blvd, MDC 02 · Tampa, FL 33612

ASSISTANT/ASSOCIATE PROFESSOR

Department of Molecular Pharmacology & Physiology
School of Basic Biomedical Sciences
University of South Florida Heath, College of Medicine

The School of Basic Biomedical Sciences within the College of Medicine at USF Health seeks outstanding scientists in the areas of molecular neuroscience, neuropharmacology or neurophysiology for two tenure/tenure earning pathway positions at the level of Assistant/Associate Professor in the Department of Molecular Pharmacology & Physiology.

USF Health has at its core the colleges of Medicine, Nursing, and Public Health. Also included are the schools of Basic Biomedical Sciences and Physical Therapy. Currently, the College of Medicine has over 540 fulltime core faculty, over 450 medical students, and over 150 students in the biomedical graduate program. In partnership with its affiliated hospitals, USF Health's research funding last year was $134 million - more than half of which came from federal sources. It has research and clinical affiliations with H. Lee Moffitt Cancer Center, the Johnnie B. Byrd Sr. Alzheimer's Research Institute, the Tampa General Hospital, the All Children's Hospital, the Shriner's Hospital for Children, and the James A. Haley and Bay Pines VA Medical Centers. USF is one of only 95 public and private universities in the U.S. that have been designated as Carnegie Comprehensive Doctoral Research University/Very High Research Activity.

Minimum requirements for Assistant Professor include a MD, PhD, or MD/PhD with a minimum of two years of experience as a junior faculty and evidence of continued growth as an educator, mentor, and a productive researcher. For Associate Professor, a minimum of five years of continuous and productive accomplishment as an Assistant Professor at a university, or an equivalent research institution is required. The successful candidate is expected to have a distinguished record of scholarly activity, NIH R01 and other extramural funding and teaching experience in a medical/graduate curriculum. A legacy of building interdisciplinary programs and experience with successfully mentoring graduate and medical students and postdoctoral-fellows is desired.

Applicants should submit, by email, a letter summarizing their qualifications and interests in the position, future research plans, updated curriculum vitae and the names and contact information of five professional references. Completed applications must be submitted to **Ms. Vanessa Ayer (vayer@health.usf.edu).** All inquiries and applications will be treated confidentially. Competitive start-up packages and salaries will be provided commensurate with experience. Review of applications will begin October 1, 2006. EOE

50 YEARS CELEBRATING SUCCESS

COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK

Neuroscience Faculty Recruitment

The Neuroscience Initiative at Columbia University is recruiting faculty with interests in the analysis of neural circuitry through molecular, genetic, cellular electrophysiological, and/or imaging approaches. We are keen to attract individuals whose research program explores neural circuits in genetically tractable model systems and in the context of well-defined behaviors. We encourage applications for positions at the Assistant Professor level but will also consider applications from more senior investigators for positions at the level of Associate or full Professor.

Columbia University currently has a world-renowned program in neurobiology and behavior, and the Neuroscience Initiative aims to enhance interactions between basic and clinical neurosciences and link the neurosciences to other scientific disciplines within the University. Faculty will be affiliated with the Center for Neurobiology and Behavior, and there will be opportunities for strong ties with scientific departments and programs on the Morningside Heights campus.

Applications for this round of recruitment are requested by November 17, 2006. A CV, cover letter including statement of interests, and three letters of reference under separate cover should be e-mailed care of Dr. Sarah Caddick, dgl2102@columbia.edu. In addition, please mail a hard copy of these documents to:

Chair, Neuroscience Search Committee
c/o: Dr. Sarah Caddick
Columbia University
Hammer Health Sciences Center
Room 2-205G
701 West 168th Street
New York NY 10032

Columbia University takes affirmative action to ensure equal employment opportunity.

POSITIONS OPEN

SENIOR RESEARCH SCIENTIST FOR CHEMISTRY
U.S. Army Research, Development and Engineering Command
U.S. Army Edgewood Chemical Biological Center
Aberdeen Proving Ground, Maryland

The U.S. Army Edgewood Chemical Biological Center seeks a Senior Scientist to conduct research in chemical defense. Primary areas of interest are reaction mechanisms, reaction kinetics, advanced analytical chemistry, and molecular recognition technologies. The successful candidate will conduct targeted laboratory studies to fill critical information gaps, including characterization of agent physical and chemical properties; development of toxicological data; and development of agent fate data. The application of this research includes detection and decontamination of chemical agents. Incumbent will select areas of investigation in fundamental mechanistic chemistry, reaction dynamics, or related aspects of experimental physical chemistry. The position requires a Ph.D in chemistry, preferably physical chemistry, or equivalent experience and must possess expertise in analytical methodology with substantial background in relevant research. Candidates must be able to work independently as a Senior Scientist. Indepth research and some nanotechnology experience and demonstrated creativity and productivity through patents, inventions, and/or significant publications are essential. *Applicants for this position must obtain and maintain a Secret Security clearance. U.S. citizenship required.* Salary commensurate with experience (Scientific and Technical $129,024 to $152,000). For detailed vacancy announcement, including specific qualification and application requirements, refer to announcement number DA-06-0 at **website: http://www.usajobs.gov.** Deadline for application is 25 October 2006. *The U.S. Army is an Equal Opportunity Employer.*

FACULTY POSITION
University of Minnesota
Department of Genetics, Cell Biology, and Development

The Department of Genetics, Cell Biology, and Development (GCD) is conducting a search for faculty, at any level, working in the general area of mobile DNA, including transposable elements, chromosome rearrangement, gene therapy, and insertional mutagenesis. The Department will devote a competitive salary, startup package, and modern laboratory space, with access to state-of-the-art core facilities in genomics/proteomics, transgenics, stem cell technology, tissue procurement, flow cytometry, statistics, analytical chemistry, and cell therapy. The candidate must have a Ph.D. or M.D., at least three years of postdoctoral experience, with evidence of high quality research productivity. Emphasis will be placed on the potential for interaction with existing research programs in GCD, particularly the members of the Arnold and Mabel Beckman Center for Transposon Research **(website: http://beckmancenter.ahc.umn.edu/).** The person selected will be expected to develop an independent, funded research program and participate in the teaching mission of the Department.

Individuals interested in this position should apply online **(website: https://employment.umn.edu).** Search for requisition number 143261. Attach curriculum vitae and a brief statement of current and future research. Three letters of reference should be mailed to: **GCD Mobile DNA Faculty Search, c/o Mary Muwahid, University of Minnesota, Department of Genetics, Cell Biology and Development, 6-160 Jackson Hall, 321 Church Street S.E., Minneapolis, MN 55455 (e-mail: muwah001@umn.edu).**

Applications will be accepted until the position is filled.

The University of Minnesota is an Equal Opportunity Educator and Employer.

CHAIR AND PROFESSOR OF BIOLOGY
Department of Biology
University of Kentucky

We seek a scholar of outstanding achievement to lead the Biology Department at the University of Kentucky in a significant expansion of its faculty, facilities, and mission. This initiative to enhance our prominence in science and education is propelled by a major legislative mandate to augment the state's flagship University. The Lexington campus offers a highly collaborative life science community with complementary disciplines represented in the Colleges of Arts and Sciences, Agriculture, Allied Health, Medicine, and Pharmacy. In addition to vigorous commitments to frontline research and graduate training, the Department of Biology provides a thorough and diverse undergraduate experience to the largest academic major on campus. Visit the Departmental **website:http://www.as.uky.edu/Biology** for additional information.

Candidates should have distinguished records of extramurally funded research and scholarly achievements commensurate with appointment at the **FULL PROFESSOR** level. Strong commitments to undergraduate and graduate education, as well as excellent communication, leadership, and administrative skills are expected. Applications should include curriculum vitae, names of at least three references, and descriptions of research interests, educational vision, leadership philosophy, and administrative experience. Materials should be addressed to the: **Search Committee Chair, Department of Biology, 101 Thomas Hunt Morgan Building, University of Kentucky, Lexington, KY 40506,** or may be submitted electronically to **e-mail: biochairsearch@uky.edu.** Review of applications will commence November 1, 2006, and continue until a suitable candidate is selected. *An Affirmative Action/Equal Opportunity Institution.*

NEUROSCIENCE
TWO TENURE-TRACK
ASSISTANT PROFESSORS

The Neuroscience & Behavior Program at Barnard College, Columbia University, seeks two tenure-track Assistant Professors, one in animal behavioral neuroscience (primary appointment in Psychology) and another in cellular neuroscience (primary appointment in Biology). Candidates should provide evidence of excellence in research and teaching, and are expected to establish an active, externally funded research program. Post-doctoral experience is preferred. Teaching responsibilities are 4 courses per year, including an upper-level seminar.

Send statement of research and teaching interests, CV, reprints, and letters of reference to: **Neuroscience Search, Department of [Psychology or Biology], Barnard College, Columbia University, 3009 Broadway, New York, New York 10027-6598** (email applications for the Cellular Neuroscience position can be sent to **biologyjob@barnard.edu**). Review of applications will begin on **October 15, 2006**.

Barnard is an Equal Opportunity Employer and encourages applications from women and individuals of diverse racial, ethnic and cultural backgrounds.

University of California
LAWRENCE LIVERMORE NATIONAL LABORATORY
Science in the National Interest

LAWRENCE POSTDOCTORAL FELLOWSHIP

The Lawrence Livermore National Laboratory (LLNL) has openings available under its Lawrence Fellowship Program. This is a highly desirable, prestigious postdoctoral position with ample resources and freedom to conduct cutting-edge research in a field of the candidate's choice. The duration of the Fellowship is up to three years. Typically two to four openings are available each year. Fellowships are awarded only to candidates with exceptional talent, credentials and a track record of research accomplishments.

Candidates will do original research in one or more aspects of science relevant to the mission and goals of LLNL which include: Physics, Applied Mathematics, Computer Science, Chemistry, Material Science, Engineering, Environmental Science, Atmospheric Science, Geology, Energy, Lasers and Biology. Successful candidates may participate in experimental or theoretical work at LLNL, and will have access to LLNL's extensive computing facilities, specialized laboratory facilities and field equipment. A senior scientist will serve as a mentor to each of the Fellows. The candidates will receive full management and administrative support. The salary is $7,933/mo.

Please refer to our web page **http://fellowship.llnl.gov** for eligibility requirements and instructions on how to apply. When applying and prompted, please mention where you saw this ad. The deadline for application is November 1, 2006. LLNL is operated by the University of California for the National Nuclear Security Administration/Department of Energy. We are an Equal Opportunity Employer with a commitment to workforce diversity.

Lawrence Livermore National Laboratory

http://fellowship.llnl.gov

Audubon

Vice President and Chief Scientist
National Audubon Society

Audubon, one of the hemisphere's premier conservation organizations, seeks a seasoned leader who is recognized internationally in the field of ornithology to shape Audubon's strategic approach to conservation and guide the organization's science programs. This is a high-paced, exciting, leadership position, requiring superior interpersonal skills and at least 10 years of progressively responsible senior level management experience in the non-profit sector. The Chief Scientist will promote the expansion of citizen science and citizen stewardship initiatives to engage local, national and international stakeholders in strategic conservation activities. The ability to integrate and align Science activities and programs with those of Audubon's public policy and education efforts is highly desired. Advanced degree in Ornithology, Conservation Biology, or Natural Resource Management with an emphasis on birds is required. Nationwide field experience and an understanding of both the practical and theoretical realms of conservation planning throughout the hemisphere are strongly desired. The ability to effectively articulate the significance of data and other scientific and technical information for Audubon's membership and the general public is essential.

Position will be based in Washington DC. Frequent travel required. For complete job description, see our website at **www.Audubon.org**.

Send resume, cover letter, and salary history to: **Seniorpositions@audubon.org**. Applications are encouraged by **October 27, 2006** but will be accepted until the position is filled.

School of Electrical Engineering and Computer Science
Washington State University, Pullman, WA
Bioinformatics/Computational Biology Faculty Position

The School of Electrical Engineering and Computer Science at Washington State University invites applications and nominations for a tenure-track position in bioinformatics/computational biology to begin January 2007 or later, at the level of assistant professor. This position is part of a university-wide initiative to strengthen its programs in the application of computational approaches to various areas of biological science. Areas of research interest include but are not limited to development of statistical, theoretical, or computational approaches for interpreting biological, health, or medical data; mathematical models or computational techniques for the study of biological systems; or quantitative strategies for integrating diverse types of biological information and developing higher-level understanding of complex systems.

Successful candidates will be expected to develop and maintain a vigorous research program supported by extramural funding, to train graduate students, and to participate in graduate and undergraduate teaching. **Required:** Earned doctorate in Computer Science or related field by January 2007 and a record of research accomplishments in bioinformatics or computational biology. **Desired:** Ability to communicate effectively with both students and colleagues, record indicating outstanding abilities and potential in research and teaching, and interdisciplinary research experience. Screening will begin **October 17, 2006**.

Qualified individuals are encouraged to send a letter of application addressing qualifications, a curriculum vitae, a statement of current and long-term research interests, and a statement of teaching experience and interests. Also arrange for four letters of reference that address research potential, teaching, and communication skills. Materials should be sent to: **B/CB Search Committee Chair, School of Electrical Engineering and Computer Science, Washington State University, PO Box 642752, Pullman, WA 99164-2752** or by email (PDF or text documents) to **robbinsj@eecs.wsu.edu**. Full Notice of Vacancy can be viewed at: **http://www.hrs.wsu.edu/employment/FAPvacancies.asp?searchin=Faculty** (**Search #4344**).

EEO/AA/ADA

POSITIONS OPEN

DEPARTMENT CHAIR
Baylor University
Department of Chemistry and Biochemistry

The Department of Chemistry and Biochemistry at Baylor University invites applications and nominations for the position of Department Chair beginning in August 2007. This position will be filled at the **ASSOCIATE or FULL PROFESSOR** rank, and is open to applicants in any area of chemistry or biochemistry.

Baylor University is a private University chartered in 1845 with an enrollment of 14,000 students. The Department of Chemistry and Biochemistry has 16 tenured and tenure-track faculty members along with eight full-time Lecturers, and offers B.A., B.S., M.S., and Ph.D. degrees. The Department currently serves approximately 275 undergraduate majors and 50 graduate students, and occupies a significant portion of the new 500,000 square feet Baylor Sciences Building. Please visit **website: http://www.baylor.edu/chemistry/chairsearch** for further details regarding the Department, Baylor University, and Waco, Texas.

Complete applications should include current curriculum vitae, a description of research interests, and a statement describing the candidate's interests and goals in seeking this position. Applications will be reviewed beginning December 1, 2006. To ensure full consideration an application should be received by January 1, 2007, but applications will be accepted until the position is filled.

Applicants should have a record of high quality research and demonstrated success in obtaining external funding. Previous administrative experience is desirable. The successful applicant will be committed to excellence in both graduate and undergraduate education and be strongly supportive of Baylor's distinctive mission and vision.

Baylor is a Baptist University affiliated with the Baptist General Convention of Texas. *As an Affirmative Action/Equal Employment Opportunity Employer, Baylor encourages minorities, women, veterans, and persons with disabilities to apply.*

Send all materials to: **Dr. Kevin Klausmeyer, Search Committee Chair, One Bear Place 97348, Waco, TX 76798: E-mail: kevin_klausmeyer@baylor.edu.**

ANALYTICAL CHEMISTRY or MATERIALS CHEMISTRY

The Department of Chemistry at the University of Michigan invites applications for an anticipated position in the area of analytical chemistry or materials chemistry at the rank of **ASSISTANT or ASSOCIATE PROFESSOR** with a proposed start date of September 1, 2007. This would be a University-year appointment (nine months academic salary with three months research supported salary.) Applications in all areas of analytical chemistry are encouraged, including but not limited to, bioanalytical, mass spectrometry, sensors, separations, and spectroscopy. Specific areas in materials chemistry may include but will not be limited to polymer synthesis and applications, supramolecular chemistry, inorganic and organic biomaterials, sensors, and optical or electronic materials. Interdisciplinary graduate programs at Michigan available for research collaborations include applied physics, biophysics, and macromolecular science and engineering. Detailed information regarding the electronic application process and required materials is available online at **website: http://www.chem.lsa.umich.edu/chem/facultyrecruit/.** The position will remain open until filled but preference will be given to applicants who have submitted all requested materials prior to October 15, 2006. Information about the Chemistry Department is available on the **website: http://www.umich.edu/~michchem.** Questions about the applications process should be sent to **e-mail: chemfac05@umich.edu.** *Women and minorities are encouraged to apply. The University of Michigan is supportive of the needs of dual-career couples and is a nondiscriminatory, Affirmative Action Employer.*

POSITIONS OPEN

DIVERSA

CAREER OPPORTUNITIES

We have exciting opportunities in the following areas:

- Research & Development
- Quality Assurance
- Formulations
- Process Development

Diversa Corporation is located in sunny San Diego, California. To review our technology and excellent benefits, visit our website at http://www.diversa.com.

FACULTY POSITION
Coastal Salt Marsh Plant Ecology

The Department of Biological Sciences at California State University, Long Beach (CSULB), invites applications for a tenure-track position beginning in fall 2007. **ASSISTANT PROFESSOR** preferred; exceptional candidates may be considered for **ASSOCIATE PROFESSOR.**

Ph.D. in biology, botany, or marine biology with research experience in coastal salt marsh plant ecology required. Candidates must have a record of published research, the ability to develop and sustain an externally funded research program involving students, a strong commitment to teach an undergraduate course in coastal wetlands plant ecology, plant ecology, general ecology or biostatistics, and the ability to effectively communicate with an ethnically and culturally diverse campus community. Submit a letter of application that includes a statement of research and teaching interests, curriculum vitae (with e-mail address), reprints of two relevant publications, and names and contact information of three references to: **Coastal Salt Marsh Plant Ecology Search, Department of Biological Sciences, California State University, Long Beach, CA 90840-3702. Telephone: 562-985-4807. E-mail: ssuetsug@csulb.edu.** Review of applications begins November 1, 2006, and continues until position is filled. Letters of application and resumes are acceptable via e-mail. Additional supplemental documents must be mailed hard copy. For additional information, see **website: http://www.csulb.edu/depts/biology/.** *CSULB is an Equal Opportunity Employer committed to excellence through diversity, and takes pride in its multicultural environment. CSULB actively encourages applications and nominations of all qualified individuals, particularly scholars from underrepresented groups.*

POSTDOCTORAL POSITION, Ph.D.
Cell Biology of Metastasis

Several Postdoctoral Positions are available to work collaboratively in the laboratories of **D. Cox** and **J. Condeelis** to study the role of inflammatory cells in tumor cell invasion and metastasis. These projects are part of a larger program to study the role of the tumor microenvironment in cancer metastasis.

Candidates must have a Ph.D. and experience and publications in cell biology. Experience with microscopic techniques preferred. All candidates must be available for an interview in New York City. Position is available immediately.

Salary is competitive. Please send curriculum vitae and three letters of recommendation by e-mail and hard copy to: **Dianne Cox, Ph.D., Assistant Professor, and John Condeelis, Ph.D., Professor and Director, Analytical Imaging Facility, Albert Einstein College of Medicine, Jack and Pearl Resnick Campus, 1300 Morris Park Avenue, Bronx, NY 10461. E-mail: dcox@aecom.yu.edu and condeeli@aecom.yu.edu.** *Equal Opportunity Employer.*

POSITIONS OPEN

ASSISTANT PROFESSOR POSITION
Botany

Juniata College, a highly ranked national liberal arts college of 1,400 students located in the scenic mountains of central Pennsylvania, invites applications for a tenure-track Assistant Professor position in botany to start the fall semester of 2007. The Biology Department at Juniata College **(website: http://www.juniata.edu/biology/)** is seeking applications from individuals utilizing modern techniques to address questions in plant development, physiology, ecology, or systematics. The successful candidate will be expected to conduct an active research program involving undergraduate students. Teaching responsibilities will include a laboratory course in botany, participation in the freshman biology course series, and several upper-level courses consistent with the candidate's specialization and Departmental needs. Relevant facilities include a green house, modern cell-culture and microscopy facilities **(website:http://departments.juniata.edu/biology/equipment.html),** and the Juniata College Raystown Field Station **(website:http://services.juniata.edu/station).** Juniata College is an undergraduate institution with a reputation for strong programs in the natural sciences. Research space and funds for research startup will be provided.

To apply, applicants with an earned Ph.D. and postdoctoral or teaching experience should submit one-page statements of (1) teaching experience, philosophy, and interests and (2) a description of their research program and plans for engaging undergraduates, (3) curriculum vitae, and (4) three letters of recommendation to: **Gail Leiby Ulrich, Director of Human Resources, Juniata College, 1700 Moore Street, P.O. Box P, Huntingdon, PA 16652.** Review of applications will begin November 1, 2006, and continue until the position is filled. It is the policy of Juniata College to conduct background checks.

Juniata College will take positive steps to enhance the ethnic and gender diversity on its campus. The College commits itself to this policy not only because of legal obligations, but because it believes that such practices are basic to human dignity. Affirmative Action/Equal Opportunity Employer.

ASSOCIATE/FULL PROFESSOR
Biochemistry

Applications are invited for a position in the Department of Chemistry and Biochemistry at Florida International University (FIU) in the area of biochemistry, with appointment starting in fall 2007. A Ph.D. in chemistry/biochemistry and a strong record of externally funded research are required. Candidates are expected to have vigorous, ongoing research programs. FIU is a public Research Extensive University with over 38,000 students located in west suburban Miami, with a new medical school scheduled to open in 2008. The Department of Chemistry and Biochemistry has a dynamic and growing Ph.D. program. Please see **website:http://www.fiu.edu/orgs/chemistry** for more details. Send curriculum vitae, transcripts, research plans, and three letters of reference to: **Search Committee, Department of Chemistry and Biochemistry, FIU, Miami, FL 33199.** Selection process will begin December 15, 2006. *FIU is an Equal Opportunity/Affirmative Action Employer.*

CAREER OPPORTUNITY

This unique program offers the candidate with an earned doctorate in the life sciences the opportunity to obtain the Doctor of Optometry (OD) degree in 27 months (beginning in March of each year). Employment opportunities exist in research, education, industry, and private practice. Contact the **Admissions Office, telephone: 800-824-5526** at **The New England College of Optometry, 424 Beacon Street, Boston, MA 02115.** Additional information at **website: http://www.neco.edu, e-mail: admissions@neco.edu.**

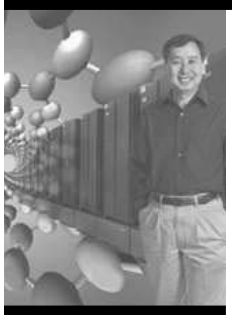
Department of Biomedical Engineering Yale University

Yale University invites applications for faculty appointments at both the junior and senior levels in its new Department of Biomedical Engineering. These faculty additions represent growth of the Faculty of Engineering and the formation of unique activities uniting Yale Engineering with the Yale School of Medicine. New faculty members will collaborate with an already strong team of engineers, basic scientists, and clinical scientists providing research and education at the undergraduate and graduate levels. The areas of specialization are open, but Yale is particularly interested in candidates with research interests in biomaterials, biosensing, and biomechanics. Successful candidates will have the potential to interact with existing foci of research in biomedical imaging, biosensing, biomechanics, drug delivery, and tissue engineering.

Please send a complete CV, example publications, research/teaching plans, and the names of three references to either of the search co-chairs: **Professor James S. Duncan or Professor W. Mark Saltzman, Yale University, Department of Biomedical Engineering, P.O. Box 208284, New Haven, CT 06520**. The deadline for applications is **December 31, 2006**.

Yale University is an Affirmative Action/ Equal Opportunity Employer and encourages applications from women and minorities.

Physicist Scientist/Engineer



Lawrence Berkeley National Laboratory (LBNL) is located in the San Francisco Bay Area on a 200-acre site in the hills above the University of California's Berkeley campus and is managed by the University. A leader in science and engineering research for more than 75 years, LBNL is the oldest of the U.S. Department of Energy's National Laboratories.

Reporting to the Division Deputy for the Scientific Support Group (SSG) of the Advanced Light Source (ALS) Division, the incumbent will function as an experimental physicist, supporting and planning experiments to meet the SSG's programmatic plans and R&D goals. The incumbent will be responsible for the operation, maintenance, and upgrades of ALS bending magnet soft x-ray beamline 9.3.2 and its associated endstations. To learn more about the ALS please visit our web site at **http://www-als.lbl.gov**.

Duties: LBNL is seeking to appoint a scientist to join a research program as applied to materials science and environmental science on bending magnet soft x-ray beamlines (9.3.2) within the SSG of the ALS. The successful applicant will develop the techniques and use of synchrotron radiation using photoemission spectroscopy, x-ray absorption spectroscopy, and ambient pressure spectroscopy for the study of electronic and atomic structure of materials. The appointee will contribute to the experimental program using any or all of the aforementioned techniques with the goal of developing an active community of ALS users of high-resolution spectroscopy in the energy range 30-1500 eV. The appointee, working closely with the ALS users, will design novel experiments and sample preparation techniques to facilitate new results in these fields, and assist in data analysis, interpretation, and simulation of results.

Qualifications: A Ph.D. in Chemistry or Physics or equivalent experience in a related field is required, in addition to a substantial published record of work in materials science and related fields using synchrotron radiation. Detailed knowledge of sample preparation, experimental setups, and data analysis for carrying out experiments in the field of photoemission, NEXAFS, and x-ray fluorescence with synchrotron radiation is essential. The candidate must also be familiar with the design and operation of a soft x-ray beamline. Demonstrated experience in the design, commissioning, and operation of high-resolution beamlines at synchrotron facilities is desired.

NOTE: This career position will be hired at the scientist level.

For fastest consideration, apply online at: **http://jobs.lbl.gov**, select "Search Jobs", and enter **019402** in the keyword search field. Enter "Science" as your source.

LBNL is an Affirmative Action/Equal Opportunity Employer committed to the development of a diverse workforce.

For more information about LBNL and its programs, visit **www.lbl.gov**.

Faculty Position
The University of California at San Diego
Section of Cell and Developmental Biology
Division of Biological Sciences
http://biology.ucsd.edu/

The Section of Cell and Developmental Biology in the Division of Biological Sciences at UCSD invites applications for a new faculty position. The appointment will be at the Assistant Professor level. Candidates pursuing innovative research in areas of developmental and cellular biology, particularly those using vertebrate experimental systems, are encouraged to apply. The successful candidate is expected to have a broad interest in development and cell biology and to complement existing strengths in the Section of Cell and Developmental Biology and the Division of Biological Sciences. The primary criteria for selection will be excellence and creativity in research and scholarship. All candidates must have a Ph.D., M.D., or an equivalent degree. The successful candidate is expected to participate in the undergraduate and graduate teaching curriculum. The level of appointment will be commensurate with qualifications and experience. Salary will be based upon University of California pay scale.

Complete applications received by **December 1, 2006** will be assured of consideration. A complete application will consist of a curriculum vitae, including a complete list of publications, a short statement of research interests and scientific goals from the applicant, and the applicant should also arrange to have three letters of recommendation (forwarded separately) to:

Development and Cell Biology Search Committee
Section of Cell and Developmental Biology
Division of Biological Sciences
Attn: Marie Stark - Mail Code 0380-B
Natural Sciences Building 1, Room 6103
University of California, San Diego
9500 Gilman Drive
La Jolla, CA 92093-0380

Applicants are welcome to include in their cover letters a personal statement summarizing leadership efforts and/or contributions to diversity. UCSD is an Equal Opportunity-Affirmative Action Employer with a strong institutional commitment to the achievement of diversity among its faculty and staff.

FLORIDA STATE UNIVERSITY

Program in Neuroscience – Pathways to Excellence Initiative
New Tenure-Track Faculty Positions

The Program in Neuroscience at the Florida State University seeks to fill five tenure-track faculty positions as part of a major initiative to expand neuroscience research. The initiative includes the development of a research quadrangle including new basic science laboratories of the College of Medicine and two new research buildings for the Departments of Biological Science and Psychology. These projects will collectively add about 490,000 square feet of space and 120 new research laboratories dedicated to biomedical, neuroscience and behavioral research.

We seek applications from noted scholars with a sustained track-record of productivity and independent support that will enhance our current strengths including motivated behaviors, sensory systems, biological rhythms, learning and plasticity, development, ingestion and metabolism, neuroendocrinology, and computational neuroscience. We invite applications for faculty positions at all ranks, although most of the positions are envisioned at the assistant professor level. Appointments will be in relevant departments. The Program in Neuroscience (**http://www.neuro.fsu.edu**) is recognized as a highly interactive, interdisciplinary program that includes 25 faculty members in 8 departments. The program has two pre-doctoral training grants and an outstanding record of productivity.

Florida State University has begun a major multi-year initiative to strengthen interdisciplinary research teams and recruit 200 new faculty campus-wide as part of our Pathways to Excellence Initiative (**http://pathways.fsu.edu**). The environment for collaboration is excellent. Located in northern Florida, Tallahassee is a beautiful, mid-sized capital city with excellent cultural and recreational opportunities.

Applicants should submit a cover letter, curriculum vitae, overview of research plans, and contact information for at least three references in a single pdf document to **search@neuro.fsu.edu**. We will begin review of applications on **October 15, 2006**.

The Florida State University is an Equal Opportunity/ Affirmative Action Employer.

POSITIONS OPEN

ASSISTANT PROFESSORSHIP in DEVELOPMENTAL BIOLOGY and ASSISTANT PROFESSORSHIP in PLANT ECOLOGY
Department of Biology
University of Missouri, St. Louis

The Department of Biology at the University of Missouri, St. Louis, invites applications for two tenure-track, Assistant Professor positions: (1) developmental biology, for which we are particularly interested in applicants who focus on nonmammalian, animal model systems and use interdisciplinary approaches, including molecular biology, cell biology, biochemistry, bioinformatics, and/or functional genomics; and (2) plant ecology, including its interface with plant physiology, plant evolution, and/or community ecology. The Department of Biology **(website: http://www.umsl.edu/~biology/)** has strong ties with the Missouri Botanical Garden and the Donald Danforth Plant Science Center. In addition, the Department houses the Whitney R. Harris World Ecology Center, established to promote international research efforts, particularly in tropical regions. Successful candidates for both positions will be expected to establish a vigorous, externally funded research program and participate in teaching and advising of both undergraduate and graduate students. Applicants must have a Ph.D. and postdoctoral experience in an appropriate discipline with evidence of high-quality research. Complete applications will include a cover letter, curriculum vitae, a concise outline of research plans, copies of up to five publications, a statement of teaching interests and philosophy, and three letters of reference. Send application documents, preferably as PDF files, to: **Maryann Hempen (e-mail: hempen@umsl.edu), Department of Biology, University of Missouri-St. Louis, One University Boulevard, St. Louis, MO 63121. Telephone: 314-516-6202; fax: 314-516-6233.** Review of applications will begin November 10, 2006, and will continue until the positions are filled. *The University of Missouri is an Affirmative Action/Equal Opportunity Employer committed to excellence through diversity.*

The Department of Biological Sciences of Northern Kentucky University (NKU) invites applications for **TWO TENURE-TRACK FACULTY POSITIONS** for fall 2007. (1) **BIOLOGICAL/EDUCATION SPECIALIST, ASSISTANT, or ASSOCIATE PROFESSOR** (commensurate with experience). Ph.D. or Ed.D. in biological sciences. Primary teaching responsibilities will include introductory biology for majors, and general biology for nonmajors. Must have knowledge of preschool through twelfth grade curricular needs and should have postsecondary teaching experience. This person will serve as a liaison with the Education Faculty and will develop outreach activities with local Teachers. Research specialization open. **(2) NUTRITIONIST, ASSISTANT PROFESSOR.** Ph.D. in nutrition with strong background in physiology or biochemistry. Postdoctoral experience is desirable. Primary teaching responsibilities will include introductory nutrition and general biology. Research specialization open. Successful candidates for both positions are expected to participate in activities of the Center for Integrative Natural Science and Mathematics **(website: http://www.cinsam.org/).** More detailed descriptions of each position plus Departmental information may be found on the **website: http://www.nku.edu/~biosci.** Send letter of application; brief statement of professional goals; statements of teaching/research philosophy; curriculum vitae; transcripts; and names, addresses, telephone numbers, and e-mail addresses of three references to: **(Name of position) Search Committee, Department of Biological Sciences, SC 204E, Northern Kentucky University, Highland Heights, KY 41099.** All application materials must be received by November 1, 2006. *NKU is an Affirmative Action/Equal Opportunity Employer and actively seeks applications from minorities and women. Upon a contingent offer of employment all applicants will be required to undergo a pre-employment criminal background check as mandated by state law.*

POSITIONS OPEN

UIC University of Illinois at Chicago

ASSISTANT OR ASSOCIATE PROFESSOR
Structural Biology

The Center for Pharmaceutical Biotechnology and the Department of Medicinal Chemistry and Pharmacognosy invite tenure-track faculty applications using structural biology approaches in areas complementary to Center and Department strengths that include both high-profile initiatives in drug discovery and innovative research programs at the interface of chemistry and biology. Ph.D. and postdoctoral experience required. Responsibilities include developing a strong, externally funded research program, and teaching in graduate and professional programs. Candidates at the Associate level must have a strong record of extramural funding. Successful candidate will have joint appointments within the Center and the Department, with extensive collaborative opportunities in a major health sciences center. Forward curriculum vitae, description of research interests, and three reference letters to: **Dr. M. Johnson, Director, Center for Pharmaceutical Biotechnology, College of Pharmacy, University of Illinois at Chicago, 900 S. Ashland, M/C 870, Chicago, IL 60607-7173.** For fullest consideration, please submit all materials by November 1, 2006.

The University of Illinois is an Affirmative Action/Equal Opportunity Employer.

AVIAN MICROBIOLOGY
Animal and Food Sciences

Applications are invited for a tenure-track faculty position at the **ASSISTANT or ASSOCIATE PROFESSOR** level. The position is 70 percent research/30 percent teaching and requires a Ph.D. with postdoctoral experience. The successful candidate will be expected to develop an extramurally funded research program in avian pathogenic microbiology focused on the study of viruses or bacteria of economic importance to the poultry industry. The individual will be responsible for advising undergraduate and graduate students and is expected to participate in the University's nationally recognized undergraduate student research program. Information about the Department and the University of Delaware can be obtained at **website: http://www.udel.edu.** Applicants should submit a letter of application, curriculum vitae, and a statement of research and teaching interests, and arrange to have three professional letters of reference sent to: **Dr. Calvin Keeler, Department of Animal and Food Sciences, University of Delaware, Newark, DE 19716-2150.** Applications may also be submitted electronically to **e-mail: ckeeler@udel.edu.** Application deadline is November 15, 2006. All application materials shall be shared with departmental faculty.

The University of Delaware is an Equal Opportunity Employer which encourages applications from minority group members and women.

The Department of Ecology and Evolutionary Biology, Tulane University, invites applications for **TWO TENURE-TRACK POSITIONS,** one in wetlands ecology and one in global change biology. One will be filled at the junior level and one at the senior level. We invite applications at all levels for each position. See **website: http://www.tulane.edu/~ebio/News/positions.htm** for more details. Send curriculum vitae, statements of research and teaching interests, selected publications, and names and addresses of three references to: **Wetlands Ecologist Search** or **Global Change Biologist Search, Department of Ecology and Evolutionary Biology, 310 Dinwiddie Hall, Tulane University, New Orleans, LA 70118-5698.** Review of applications will begin December 1, 2006, and the searches will remain open until the positions are filled. These positions are subject to a final University determination on funding. *Tulane University is an Affirmative Action/Equal Employment Opportunity Employer.*

POSITIONS OPEN

ASSOCIATE DIRECTOR FOR RESEARCH

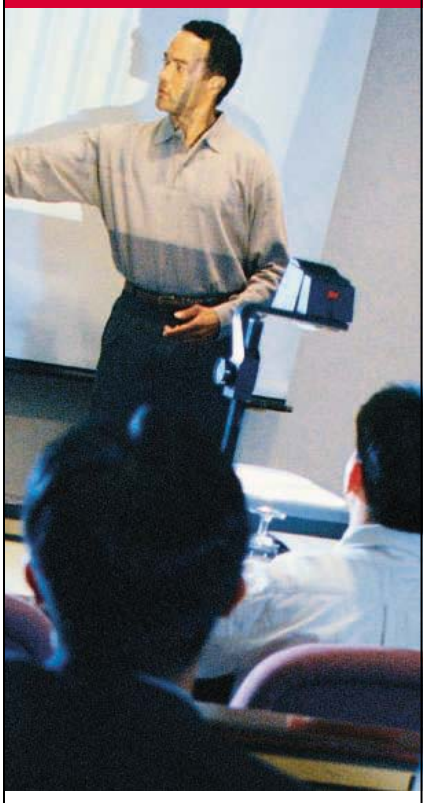
Indiana University (IU) School of Medicine, Medical Sciences Program in Bloomington, Indiana, is seeking a leading Ph.D., M.D., or M.D./Ph.D. in cancer biology to become the Associate Director for Research; this is a tenure-track position. The Medical Sciences Program is presently expanding its research efforts by recruiting several Basic/Translational Scientists. The successful applicant will have a well-established federally funded research program and will be expected to develop and direct an active Cancer Biology Program that builds on existing collaborations between Medical Sciences Faculty and others from the College of Arts and Sciences (biology, biochemistry, chemistry, neurosciences, physics) in Bloomington and with the established cancer researchers of the IU School of Medicine in Indianapolis as outlined in the Indiana University Life Sciences Strategic Plan **(website: http://lifesciences.iu.edu).** The Associate Director for Research will be expected to maintain a federally funded research program, lead and coordinate new faculty recruitment, assist existing programs in expanding their funding base, and develop new research programs relevant to cancer biology. The Associate Director is also expected to strengthen existing ties with the NCI-designated Cancer Research Institute, the Indiana Genomics Initiative, and the Indiana Metabolomics and Cytomics Initiative. Outstanding scholarly achievements, a deep commitment to academic excellence, strong leadership and administrative skills, proven teaching skills, and a vision for basic science and translational research in an academic setting are expected. Additional information may be obtained from **website: http://medsci.indiana.edu.** A review of applications will continue until the position is filled. Full consideration is assured for applications received by October 15, 2006. Applicants should send their curriculum vitae; a statement of current and future research activities and training philosophy; and the names of four references as a single PDF file to **e-mail: bevhill@indiana.edu.** Additional materials may be mailed to: **Associate Director Search Committee, Indiana University School of Medicine, Medical Sciences Program, Jordan Hall 105, 1001 East 3rd Street, Bloomington, IN 47405.**

Equal Employment Opportunity/Affirmative Action Employer, Minorities/Females/Persons with Disabilities.

The Department of Chemistry and Biochemistry at the University of Oklahoma invites applications for a tenure-track teaching emphasis faculty position at the rank of **ASSISTANT PROFESSOR** in the area of biochemistry. Applicants must have completed the Ph.D. degree by the beginning of the appointment, August 16, 2007. The successful candidate will be primarily responsible for excellent teaching of biochemistry at the undergraduate level consisting of two three-credit hour lecture courses per semester in addition to responsibility for the undergraduate biochemistry laboratory program. Although primarily a teaching appointment, the successful candidate will be expected to establish an active research program involving undergraduates or develop innovative teaching techniques and methods or other forms of significant creative activity relevant to the area of biochemistry. The successful candidate can expect appropriate service assignments such as advising undergraduate majors. Candidates should submit curriculum vitae, a statement of teaching experience, interests and philosophy, and a detailed description of plans for research or other creative activity. Candidates should also arrange to have three letters of recommendation submitted directly to the **Chair of the Search Committee**. Application materials should be sent to: **Professor Paul F. Cook, Chair of Teaching Emphasis Biochemistry Faculty Search Committee, Department of Chemistry and Biochemistry, 620 Parrington Oval, University of Oklahoma, Norman, OK 73019.** We will also accept completed applications in PDF format sent to **e-mail: sgfisher@ou.edu.** The review of applications will begin on November 15, 2006, and continue until the position is filled. *Minorities and women are especially encouraged to apply. The University of Oklahoma is an Affirmative Action/Equal Opportunity Employer and is responsive to the needs of dual-career couples.*

Faculty Careers 3

A *Science* Advertising Supplement



Be sure to read this special ad supplement devoted to faculty career opportunities in the **13 October issue of *Science*.**

Find faculty position listings and career resources online at **www.sciencecareers.org.**

For advertising information, contact:

U.S. Daryl Anderson
phone: 202-326-6543
e-mail: danderso@aaas.org

Europe and International
Tracy Holmes
phone: +44 (0) 1223 326 500
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Japan Jason Hannaford
phone: +81 (0) 52 757-5360
e-mail: jhannaford@sciencemag.jp

Rat der Eidgenössischen Technischen Hochschulen ETH-Rat | Conseil des écoles polytechniques fédérales CEPF | Consiglio dei politecnici federali CPF | Cussegl da las scolas politecnicas federalas CSPF | Board of the Swiss Federal Institutes of Technology ETH Board

The ETH Board wishes to appoint a

Director of WSL

in Zurich, Switzerland. The Swiss Federal Institute for Forest, Snow and Landscape Research WSL (www.wsl.ch) is part of the ETH Domain, and focuses on the use and protection of landscapes and habitats. It is home to an international team of approx. 400 scientists and staff. WSL engages in innovative science at the interface of environmental engineering and the natural and social sciences. It links pure theoretical science with the practical implementation of scientific findings to provide key recommendations for sustainable environmental policies in Switzerland.

The Director will be an innovative, open-minded, charismatic, decisive, and cosmopolitan personality with high-ranking, internationally acknowledged academic qualifications. He or she will have a distinguished record as a scientist, and will have shown, through previous appointments, clear leadership in the scientific, technological, and societal realms. The Director of WSL must be ready to take independent decisions and be able to actively integrate all aspects of the institution, and thereby create a functional yet non-hierarchical management culture. The personality to be elected must have excellent communication skills with regard to the public and the academic community, excellent knowledge of English, and a very good command of German, French or Italian.

The successful candidate will be affiliated to the Federal Institute of Technology at Zurich or Lausanne. The ETH Domain is an equal opportunity employer.

Please send your written application with extended curriculum vitae marked «Private and Confidential» by October 20, 2006 to: Prof. Alexander Zehnder, President of the ETH Board, Häldeliweg 15, 8092 Zürich. Your application will be treated with the strictest confidence. For further details contact Dr. Klara Sekanina, phone +41 44 632 20 64, or sekanina@ethrat.ch.

Framingham State College

Assistant Professor - Biology, Coordinator of Biology Secondary Education

The Biology Department at Framingham State College seeks applicants for a tenure-track position starting in September 2007. The successful applicant will be a broadly trained biologist with experience in secondary education and/or teacher preparation. Responsibilities include professional preparation and oversight of student teachers as well as teaching introductory biology courses and courses in the area of applicant's expertise. Minimum qualifications include a Ph.D. in Biology and high school and college-level teaching experience.

Send curriculum vitae, transcripts, statement of teaching philosophy and research interests, and the names of three references by 12/1/06, to: **Margaret Carroll, Chair, Department of Biology, Framingham State College, P.O. Box 9101, Framingham, MA 01701-9101; e-mail: mcarrol@frc.mass.edu**.

Framingham State College is an Affirmative Action/Equal Opportunity Employer. Applications are especially encouraged from women, people of color and persons with disabilities.

NEUROSCIENCE

As part of an ongoing expansion of Biological and Biomedical science faculty, **Kent State University** invites applications for a tenure-track position in the Department of Biological Sciences at the rank of Assistant Professor beginning in August 2007. We seek candidates with research interests in any area of neuroscience. Strengths of the department include strong interdisciplinary affiliations among the basic science units, the School of Biomedical Sciences, The Cleveland Clinic Foundation, and the Northeastern Ohio Universities College of Medicine. The department offers superb core research facilities and subsidized animal care. The successful candidate is expected to establish an extramurally funded research program and exhibit a commitment to excellence in graduate and undergraduate education. Applicants must have a Ph.D. degree and postdoctoral experience. For more information on this position and the faculty, see **www.kent.edu/biology/neuro_search.cfm**.

Applicants should send their curriculum vitae, statement of research interests, and three letters of recommendation to: **Chair, Neuroscience Search Committee, Department of Biological Sciences, Kent State University, P.O. Box 5190, Kent, Ohio 44242-0001; Fax: 330-672-3713**. Review of applications will begin **October 31, 2006**, and continue until the position is filled.

Kent State University is an Affirmative Action/Equal Opportunity Employer and encourages applications from candidates who would enhance the diversity of the University's faculty.

POSITIONS OPEN

TENURE-TRACK FACULTY POSITION
Inorganic/Materials Chemistry
Florida State University

The Department of Chemistry and Biochemistry at Florida State University anticipates an opening for a tenure-track **ASSISTANT PROFESSOR** position in the area of inorganic and materials chemistry to begin in fall 2007. Candidates are expected to demonstrate the ability to establish a highly creative interdisciplinary research program that is externally funded, and to contribute to the graduate and undergraduate teaching in the inorganic and materials program in the Department. The applicant's research interests can be drawn from any interdisciplinary materials/inorganic chemistry sub-area, including: biomaterials, nanoscience, energy, catalysis, bioinorganic chemistry, solid-state chemistry, organometallics, and/or development of state-of-the-art spectroscopic tools or methods applied to these areas. Applicants should send curriculum vitae, a description of their research plans, a statement of teaching philosophy and teaching interests, and arrange to have three letters of recommendation sent to the: **Chair of the Inorganic/Materials Search Committee, Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL 32306-4390.** Applications should be received by November 17, 2006, to ensure full consideration. *Florida State University is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.*

DIRECTOR
National Center for Water Quality Research

Heidelberg College invites applications for the position of Director of its internationally recognized National Center for Water Quality Research (NCWQR). We seek an energetic Director who can guide the continued operation and expansion of NCWQR programs. The NCWQR's mission is to support the sustainable use of our nation's water resources and the protection of human health and ecological integrity through improved water quality. The NCWQR moved into the new Gillmor Science Hall in 2005. Applicants must have experience in water resources or a related environmental discipline; a Ph.D. is preferred. The Director reports directly to the Vice President for Academic Affairs and, depending on qualifications, may be tenured upon appointment. A detailed description of this position and information on NCWQR programs and staff can be found at **website: http://www.heidelberg.edu/wql.** Screening of applications will begin immediately and will conclude when a suitable candidate is appointed. Applicants should include curriculum vitae and a letter explaining their qualifications for this position. Applications should be sent to: **Dr. Kenneth Krieger, Chair, NCWQR Director Search, Gillmor Hall, Heidelberg College, Tiffin, OH 44883. E-mail: kkrieger@heidelberg.edu.** *Heidelberg College is an Affirmative Action, Equal Opportunity Employer.*

THE ENDOWMENT FOR SCHOLARS
in Biomedical Research at
The University of Texas Southwestern Medical Center

University of Texas (UT), Southwestern, is pleased to announce the continuation of the Endowed Program for Scholars in Biomedical Research. The Program, which is fully funded from private endowment, will provide $1,000,000 over four years to support the research activities of each new **ASSISTANT PROFESSOR** (tenure track) appointed to the Program; five will be appointed annually. Academic appointments and research space will be provided by individual medical school departments or research centers. Positions in both basic science and clinical departments are available. The goal of the program is to assure a successful beginning of the research careers of an ever-growing cadre of outstanding young investigators at UT Southwestern.

For detailed information about currently available faculty positions, please access our webpage **website: http://www8.utsouthwestern.edu/utsw/home/scholars/index.html.**

UT Southwestern is an Equal Opportunity Institution.

FACULTY POSITIONS (TWO) IN BIOLOGY

York College of the City University of New York invites applications for two tenure-track positions at the **ASSISTANT PROFESSOR** level in genetics/bioinformatics and in plant physiology/evolution to begin September 1, 2007. Qualifications include a Ph.D. with postdoctoral experience and evidence of excellence in teaching. Instructional responsibilities include lecture and laboratory courses in area of expertise as well as other major or nonmajor courses as needed. Candidates must demonstrate a strong interest and commitment to undergraduate teaching and the capability of developing and maintaining an active research program supported by external funding. The academic program and instructional and research equipment available at York College can be found at **website: http://natsci.york.cuny.edu.** Applicants should submit a cover letter, curriculum vitae, statements of research and teaching experience, and the name and contact information of three professional references to: **Dr. Margaret MacNeil, Biology, York College/CUNY, 94-20 Guy R. Brewer Boulevard, Jamaica, NY 11451.** The application deadline is October 23, 2006. *Equal Employment Opportunity/Affirmative Action/ADA/IRCA.*

FACULTY POSITIONS, TENURE TRACK
Department of Psychiatry

The Department of Psychiatry and the Center for the Study of Traumatic Stress at the Uniformed Services University of the Health Sciences (USUHS) seeks to fill tenure-track neuroscience laboratory research and teaching positions **(ASSISTANT/ASSOCIATE PROFESSOR).** The Department, twenty full-time faculty, seeks to expand ongoing neuroscience research, animal and human, in: stress; anxiety (particularly acute stress responses, posttraumatic stress disorder and dissociation); depression; behavior and drug use. Individuals who hold Ph D. or M.D. degrees and have active fundable research are invited to apply. Send curriculum vitae, description of current and anticipated research, and three references to: **Robert Ursano, M.D., Chairman, Department of Psychiatry, USUHS, 4301 Jones Bridge Road, Bethesda, MD 20814-4799 (e-mail: rursano@usuhs.mil).** Review of applications is ongoing. *The University is an Affirmative Action/Equal Opportunity Employer.*

FACULTY POSITION
Bacterial Genomics
University of Illinois at Chicago

The Center for Pharmaceutical Biotechnology, the Department of Microbiology and Immunology, and the Department of Biochemistry and Molecular Genetics invite applications for a tenure-track faculty position in a general area of microbial genomics. Responsibilities include developing a strong, externally funded research program and teaching in graduate and professional programs. Successful candidate will have joint appointments with the Center for Pharmaceutical Biotechnology and one of the Departments. Send curriculum vitae, description of research interests, and three letters of reference by November 1, 2006, to: **Dr. A. Mankin, Chair, Search Committee, Center for Pharmaceutical Biotechnology - M/C 870, University of Illinois at Chicago, 900 S. Ashland Avenue, Chicago, IL 60607-7173.**

The University of Illinois is an Affirmative Action/Equal Opportunity Employer.

RESEARCH ASSOCIATE, UNIVERSITY OF KANSAS. Investigate and apply nuclear magnetic resonance (NMR) spectroscopy to carry out structural and dynamic studies of paramagnetic and diamagnetic proteins, in particular heme oxygenase. Requires a Ph.D. and a strong background in NMR spectroscopy. For a complete description search for position 00008278 at **website: https://jobs.ku.edu** or **e-mail: bkjohnson@ku.edu.** Priority will be given to applications received by October 31, 2006. *Equal Opportunity/Affirmative Action Employer.*

FACULTY POSITION
University of California, San Diego
Chemistry/Biochemistry–Cancer Center

The Department of Chemistry and Biochemistry of University of California, San Diego (USCD), **(website: http://chem.ucsd.edu)** in collaboration with the Moores Cancer Center **(website: http://cancer.ucsd.edu/)** invites applications for a tenure-track or tenured faculty position in an area of chemistry, chemical biology, or biochemistry contributing to basic knowledge in the field of oncology. Candidates must have a Ph.D. and a demonstrated ability for creative research and teaching at the undergraduate and graduate levels. Salary commensurate with qualifications and based on University of California pay scale. Candidates should send curriculum vitae, list of publications, reprints of up to five representative papers, and a summary of research and teaching plans to: **Chair, Cancer Center Search Committee 4-792S, University of California, San Diego, Department of Chemistry and Biochemistry, Mail Code 0332, La Jolla, CA 92093-0332.** In addition, candidates should arrange for three letters of recommendation to be sent to the above address. The deadline for applications is November 17, 2006, but until the position is filled, all applications received will be assured full consideration. *UCSD is an Equal Opportunity/Affirmative Action Employer with a strong institutional commitment to the achievement of diversity among its faculty and staff.*

FACULTY POSITION in MOLECULAR, CELLULAR, and DEVELOPMENTAL BIOLOGY
University of Colorado at Boulder

The Department of Molecular, Cellular, and Developmental Biology invites applications for a tenure-track **ASSISTANT PROFESSOR** in the area of molecular, cellular, or developmental biology. Applicants must have a Ph.D., M.D., or equivalent; and postdoctoral research experience. The candidate is expected to develop a vigorous and innovative research program, and have enthusiasm for teaching at the undergraduate and graduate levels.

Applicants should submit curriculum vitae and a concise statement of research and teaching interests, and arrange to have three reference letters sent to:

Molecular, Cellular, and Developmental Biology
Faculty Search Committee
Department of MCD Biology
University of Colorado at Boulder
347 UCB
Boulder, CO 80309-0347

Review of applications will begin December 1, 2006. Applications will continue to be accepted until the position is filled. *The University of Colorado at Boulder is committed to diversity and equality in education and employment.*

FACULTY POSITION
Molecular Biology
Johns Hopkins University

The Department of Biology, Johns Hopkins University, invites applications for a tenure-track/tenured faculty appointment in the field of molecular biology. Any level will be considered. Areas of particular interest include but are not limited to genomics, microRNAs, gene expression, and viology. Candidates will be expected to establish a vigorous research program and to participate in undergraduate and graduate teaching. The committee will begin to review applications after November 15, 2006.

Please submit curriculum vitae, statements of current and planned research, and a statement of teaching interests/philosophy. Arrange to have three letters of recommendation sent to:

Chair, Search Committee
Department of Biology
Krieger School of Arts and Sciences
Johns Hopkins University
3400 N. Charles Street
Baltimore, M.D. 21218
Website: http://www.bio.jhu.edu/

Johns Hopkins University is an Equal Opportunity/Affirmative Action Employer.

Does your next career step need direction?

There's only one place to go for career advice if you value the expertise of *Science* and the long experience of AAAS in supporting career advancement - ScienceCareers.org. The pages of *Science* and our website ScienceCareers.org offer:

- Thousands of job postings
- Career advice articles and tools
- Funding information
- Networking opportunities

www.sciencecareers.org

POSITIONS OPEN

CHEMISTRY

The Department of Chemistry and the Life Sciences Institute (LSI) at the University of Michigan invite applications for an anticipated position at the rank of **ASSISTANT PROFESSOR or ASSOCIATE PROFESSOR** in the field of chemistry with a proposed start date of September 1, 2007. Any area of chemistry that overlaps with life sciences is of interest including, but not limited to, bio-analytical, biomaterials, and bio-organic. The successful candidate's laboratory will be located in the LSI, a scientific enterprise at the University of Michigan dedicated to opening new scientific paths by blending diverse research talents in a state-of-the-art collaborative facility. Candidates are expected to develop an internationally recognized program of scholarly research and to excel in teaching at undergraduate and graduate levels. Detailed information regarding the electronic application process and required material is available online at **website: http://www.chem.lsa.umich.edu/chem/facultyrecruit/.** The position will remain open until filled but preference will be given to applicants who have submitted all requested materials prior to October 15, 2006. Information about the Chemistry Department and LSI is available online **(websites:http://www.umich.edu/~michchem; www.lifesciences.umich.edu/institute/).** Questions about the application process should be sent to **e-mail: chemfac06@umich.edu.** *Women and minorities are encouraged to apply. The University of Michigan is supportive of the needs of dual-career couples and is a nondiscriminatory, Affirmative Action Employer.*

FACULTY POSITION, ASSISTANT/ ASSOCIATE OF BIOLOGY, GENOMICS

The Department of Biology at the University of Rochester is continuing its hiring initiative. It is our goal to continue to build a research and graduate education environment that encourages interactions across biological disciplines and integrative approaches to biology. This year we intend to fill a tenure-track position at either the Assistant or Associate level in genomics. Highly qualified candidates in all areas, including functional and comparative genomics, are encouraged to apply. Our research and graduate programs are integrated into a larger research campus, which includes computer sciences, brain and cognitive sciences, biomedical engineering, and the School of Medicine and Dentistry.

Please send curriculum vitae, a statement of research interests, three letters of reference, and one to two reprints to: **Faculty Search Committee, Department of Biology, University of Rochester, Rochester, NY 14627-0211.** Applications may also be submitted as PDF files to **e-mail:ynkr@mail.rochester.edu.** Review of applications will begin November 1, 2006. *The University of Rochester is an Equal Opportunity Employer.*

GENETICIST, BUCKNELL UNIVERSITY

The Biology Department at Bucknell University invites applications for an entry-level, tenure-track **ASSISTANT PROFESSOR** position to begin August 2007. We are seeking a broadly trained Geneticist who could teach an upper-level course in his/her area of specialty, contribute to our core genetics course, and participate in interdisciplinary programs. The successful candidate is expected to establish a research program that involves talented undergraduates and attracts extramural funding. Ph.D., postdoctoral experience, evidence of teaching effectiveness, and a strong research record are required. Bucknell University is a premier liberal arts university with a long-standing tradition of excellence in the sciences. Startup funds and internal funding for research are available. To apply, please refer to **website: http://www.bucknell.edu/jobs.** Review of applications will begin on October 20, 2006. The search will remain open until the position is filled. *An Equal Opportunity/Affirmative Action Employer, Bucknell University especially welcomes applications from women and minority candidates.*

ASSISTANT/ASSOCIATE PROFESSOR
Cancer Biology
Department of Pathology
University of Colorado Comprehensive Cancer Center
University of Colorado Health Sciences Center at Fitzsimons

The University of Colorado Comprehensive Cancer Center and the Department of Pathology at the University of Colorado School of Medicine invite applications for a full-time tenure-eligible position in pathology at the Assistant or Associate Professor level, commensurate with experience and accomplishments. Applicants should have a Ph.D., M.D. or M.D./Ph.D. degree, postdoctoral research experience in cancer biology or a related field, and an exceptional record of research accomplishments. Individuals with experience in the area of cancer biology including malignant transformation, cell proliferation, signal transduction, cell motility and migration, metastasis, and apoptosis are especially encouraged to apply. The successful applicant will be expected to develop a vigorous externally funded research program and contribute to the teaching mission at the School of Medicine and the Graduate School. Applicants should submit curriculum vitae, a brief description of research interests, and arrange to have three letters of reference forwarded by November 15, 2006, to: **Steven M. Anderson, Ph.D., UCDHSC Department of Pathology, Mailstop 8104, P.O. Box 6511, Aurora, CO 80045. E-mail address: pathology.researchjobs@uchsc.edu.**

The University of Colorado is committed to diversity and equality in education and employment.

The Department of Chemistry at the University of Michigan invites applications for an anticipated position at the rank of **ASSISTANT PROFESSOR or ASSOCIATE PROFESSOR** in any subdiscipline of chemistry with a proposed start date of September 1, 2007. This would be a University-year appointment (nine months academic salary with three months research supported salary). Candidates are expected to develop an internationally recognized program of scholarly research and to excel in teaching at undergraduate and graduate levels. Detailed information regarding the electronic application process and required materials is available online at **website: http://www.chem.lsa.umich.edu/chem/facultyrecruit/.** The position will remain open until filled but preference will be given to applicants who have submitted all requested materials prior to October 15, 2006. Information about the Chemistry Department is available on the **website:http://www.umich.edu/~michchem.** Questions about the application process should be sent to **e-mail: chemfac06@umich.edu.** *Women and minorities are encouraged to apply. The University of Michigan is supportive of the needs of dual-career couples and is a nondiscriminatory, Affirmative Action Employer.*

MEDICAL INFORMATION RESEARCHER/WRITER

PinnacleCare, a growing fast-paced health management company in Baltimore, Maryland, is seeking Medical Researchers/Scientists/Specialists to join our research division. The successful candidates will perform information research on cutting-edge medical science mostly among published issues of scholarly biomedical journals for the latest findings in disease mechanism, diagnosis, current treatment options, and clinical trials. You will then write easy-to-understand reports personalized for PinnacleCare members.

Candidates should have a Ph.D., preferably in a field of clinical science. The successful candidates should have the ability to read and understand technical research papers and an outstanding ability to extract key, relevant information from the literature and write reports in clear, concise, and plain English.

Please e-mail your curriculum vitae or resume to: **Benjamin Yang, M.D., Ph.D., Director of Research, e-mail: resumes@pcistaff.com. Website: http://www.pinnaclecare.com.**

INDIANA UNIVERSITY NORTHWEST
Plant Molecular Biologist

The Department of Biology at Indiana University (IU) Northwest invites applications for a tenure-track position in plant molecular biology at the level of **ASSISTANT PROFESSOR.** Candidates must have a Ph.D.; postdoctoral experience is a plus. The individual will contribute to our B.S. in biology and to redeveloping the general education curriculum, and may contribute to developing a proposed M.S. in biotechnology (e.g. any field of plant molecular biology) and/or a proposed M.S. in environmental studies (e.g. plant conservation genetics with molecular focus). Excellence in teaching at different levels is required. Northwest Indiana features the floristically diverse Indiana Dunes region lying at the junction of prairie, savanna/woodland, eastern deciduous forest, and northwoods ecoregions with diverse wetland types throughout. A 55 acre nature preserve is being restored adjacent to campus; on campus we have a tropical greenhouse. Applicants should send curriculum vitae, a statement of research interests, a statement of teaching philosophy and interests, up to three representative publications, and arrange for three letters of recommendation to be sent to: **Dr. Spencer Cortwright, Chair, Department of Biology, Indiana University Northwest, 3400 Broadway, Gary, IN 46408.** Materials to be considered for position must be in by deadline of January 2, 2007. Anticipated start date of August 2007.

One of the eight comprehensive Universities in the Indiana University system, IU Northwest is located in metropolitan Northwest Indiana, approximately 30 miles southeast of Chicago and 10 miles from the Indiana Dunes National Lakeshore. The campus has a diverse student population of 5,000 and offers Associate, Baccalaureate, and Master degrees in a variety of undergraduate and graduate programs in arts and sciences, business and economics, education, nursing and health professions, public and environmental health and social work. IU Northwest emphasizes quality teaching, research and service. As a student-centered campus, IU Northwest is committed to academic excellence characterized by a love of ideas and achievement in learning, discovery, creativity, and engagement.

IU Northwest is an Equal Employment Opportunity, Affirmative Action Employer with a commitment to recruiting and retaining a diverse faculty and staff by expanding employment opportunities for minorities, women, and persons with disabilities. Qualified candidates with an interest in developing and implementing curricula that address multicultural issues and/or with demonstrated success in working with diverse populations of students are strongly encouraged to apply.

DEVELOPMENTAL BIOLOGIST ASSISTANT PROFESSOR

The Department of Biological Sciences at Union College invites applications for a full-time tenure-track Assistant Professor position in developmental biology, to begin in September 2007. The successful candidate will teach a course in developmental biology, will participate in the introductory biology sequence, and will develop an additional course in her or his area of expertise that would complement our current course offerings. Individuals using plant or animal developmental systems in their research are encouraged to apply. A Ph.D. and strong commitment to undergraduate education is required. Postdoctoral experience is preferred. Union College is a highly selective liberal arts college with an emphasis on student research and interdisciplinary programs. Please send a letter of application with curriculum vitae, a statement detailing teaching experience and philosophy, a separate statement of research interests, and up to three peer-reviewed publications by November 22, 2006, to: **Dr. Barbara Danowski, Developmental Biology Search, Department of Biological Sciences, Union College, Schenectady, NY 12308.** *Union College is an Equal Opportunity Employer and strongly committed to student and workforce diversity.*

ANNOUNCEMENTS

POSITIONS OPEN

CALIFORNIA INSTITUTE OF TECHNOLOGY invites applications for a tenure-track professorial position in the Division of Biology. The successful applicant is expected to develop an innovative research program aimed at deciphering the molecular mechanisms that underlie biological phenomena at the level of molecules, cells, or organisms, and to be committed to high quality teaching. Preference will be given to candidates at the Assistant Professor level; however, consideration will also be given to more senior applicants. Appointment is contingent upon completion of Ph.D.

Submit on-line by **December 31, 2006** a brief cover letter, a curriculum vitae, relevant publications, and a description of proposed research. Arrange to have at least three letters of recommendation sent by email to **cellular-search@caltech.edu**. Please visit **www.biology.caltech.edu/Positions** for application submission.

The California Institute of Technology is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.

CALIFORNIA INSTITUTE OF TECHNOLOGY invites applications for a tenure-track Assistant Professor position in the area of complex biological systems in the Division of Biology. Areas of interest include (but are not limited to) quantitative and interdisciplinary studies of the dynamical behavior of biological networks (involving molecules, cells including neurons, circuits, organisms, or synthetic approaches). We are particularly interested in individuals who propose to combine computational and experimental approaches to understand emergent properties of biological systems. Joint appointments in other Divisions may be arranged as appropriate. Appointment will be contingent upon completion of all requirements for a doctoral degree and successful research experience in an appropriate area of biology, biochemistry, chemistry, neurobiology, biophysics, physics or related fields. Outstanding candidates who have strong commitments to research and teaching are encouraged to apply.

Submit on-line by **December 31, 2006** a brief cover letter, a curriculum vitae, relevant publications, and a description of proposed research. Arrange to have at least three letters of recommendation sent by email to **complex-sys-search@caltech.edu**. Please visit **www.biology.caltech.edu/Positions** for application submission.

The California Institute of Technology is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.

INTEGRATIVE PHYSIOLOGY

As part of an ongoing expansion of the Biological sciences faculty, **Kent State University** invites applications for a tenure-track position in the Department of Biological Sciences at the rank of Assistant or Associate Professor beginning in August 2007. We seek candidates with research interests that address physiological questions from the molecular to organismal levels of analysis. Strengths of the department include strong interdisciplinary affiliations among the basic science units, the School of Biomedical Sciences, The Cleveland Clinic Foundation, and the Northeastern Ohio Universities College of Medicine. The department offers superb core research facilities and subsidized animal care. The successful candidate is expected to establish an extramurally funded research program and exhibit a commitment to excellence in graduate and undergraduate education. Applicants must have a Ph.D. degree and postdoctoral experience. For more information see **www.kent.edu/biology/phys_search.cfm**.

Applicants should send their curriculum vitae, statement of research interests, and three letters of recommendation to: **Chair, Physiology Search Committee, Department of Biological Sciences, Kent State University, P.O. Box 5190, Kent, Ohio 44242-0001; Fax: 330-672-3713.** Review of applications will begin **October 31, 2006**, and continue until the position is filled.

Kent State University is an Affirmative Action/Equal Opportunity Employer and encourages applications from candidates who would enhance the diversity of the University's faculty.

POSITIONS OPEN

EVOLUTIONARY BIOLOGIST

The Biology Department of Carthage College invites applications for a **TENURE-TRACK POSITION** in biology to teach introductory biology and courses in the candidate's area of interest. Candidates must hold the Ph.D. degree in biology or a related field at time of appointment. We are seeking an individual with a strong commitment to teaching and involving undergraduates in research. In addition to formal scholarly credentials, candidates must have enthusiasm for teaching and undergraduate research in a small college atmosphere. Carthage faculty members also teach general education courses regularly, including Heritage seminars, the College's core curriculum.

Salary and benefits are fully competitive. Rank of appointment is dependent upon qualifications.

Located on the shore of Lake Michigan, midway between Milwaukee and Chicago, Carthage offers quick urban access from the relaxed environment of a small city. Founded in 1847, Carthage is affiliated with the Evangelical Lutheran Church in America. Additional information on Carthage and this position is available at **website: http://www.carthage.edu/careers.**

Applications including current curriculum vitae, a statements of teaching philosophy and research interests, and three letters of recommendation should be sent to: **Dr. Kevin Crosby, Chair, Division of Natural Sciences, Carthage College, 2001 Alford Park Drive, Kenosha, WI 53140-1994.** Applications should be received by November 15, 2006. *Carthage College values diversity.*

CHAIR AND PROFESSOR
College of Medicine
Department of Physiology

The University of Tennessee Health Science Center invites applications for the position of Chair in the Department of Physiology. This is a position at the level of Professor.

Applicants should hold the Ph.D. degree or equivalent. Duties include serving as Chair of a Department that has consistently ranked among the top 10 Physiology Departments in the country in extramural funding. The Department has 19 tenure-track faculty. The successful candidate must have a strong commitment and history to excellence in research, medical education, and scholarly productivity. Administrative experience is desirable. The University has an excellent benefits package. All qualified applicants should submit a letter of application, complete curriculum vitae and three letters of recommendation to:

Russell W. Chesney, M.D.
Fellow of the American Academy of Pediatrics
Chair, Physiology Chair Search Advisory Committee
P. O. Box 63647
Memphis, TN 38163

The University of Tennessee is an Equal Employment Opportunity/Affirmative Action/Title VI/Title IX/Section 504/ADA/ADEA Institution in the provision of its education and employment programs and services.

SMITHSONIAN INSTITUTION
Fellowship Program

GRADUATE STUDENT, PREDOCTORAL, POSTDOCTORAL, and SENIOR FELLOWSHIPS in animal behavior, ecology, and environmental science; including an emphasis on the tropics; Earth sciences and paleobiology; evolutionary and systematic biology; history of science and technology. Tenable in residence at the Smithsonian facilities. Stipends and tenure vary. Awards are contingent upon the availability of funds.

Deadline: January 15 annually. Contact: **Office of Research Training and Services, Smithsonian Institution, Desk S, P.O. Box 37012, Victor 9300 MRC 902, Washington, D.C. 20013-7012, telephone: 202-275-0655, e-mail: siofg@si.edu, website: http: //www.si.edu/research+study.**

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POSITIONS OPEN

MICROBIOLOGIST

The Biology Department of Carthage College invites applications for a **TENURE-TRACK POSITION** in biology to teach introductory biology, microbiology, and courses in the candidate's area of interest. Candidates must hold the Ph.D. degree in biology or a related field at time of appointment. We are seeking an individual with a strong commitment to teaching and involving undergraduates in research. In addition to formal scholarly credentials, candidates must have enthusiasm for teaching and undergraduate research in a small college atmosphere. Carthage faculty members are also expected to teach general education courses regularly, including Heritage seminars, the College's core curriculum.

Salary and benefits are fully competitive. Rank of appointment is dependent upon qualifications.

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Applications including current curriculum vitae, statements of teaching philosophy and research interests, and three letters of recommendation should be sent to: **Dr. Kevin Crosby, Chair, Division of Natural Sciences, Carthage College, 2001 Alford Park Drive, Kenosha, WI 53140-1994.** Applications should be received by November 15, 2006. *Carthage College values diversity.*

A **POSTDOCTORAL FELLOWSHIP** is available at the University of Alabama, Birmingham, for an NIH-funded project to study the interaction of anesthetics with proteins. The project will employ state-of-the-art molecular techniques to identify the structural basis for anesthetic-protein interactions and the subsequent effect of the anesthetic on the protein structure, function, and dynamics. The long-term objective of the project is to integrate structural biology with physiological studies to elucidate mechanisms of anesthetic action. A considerable amount of preliminary data has already been gathered which offers a unique opportunity for a competent researcher. The ideal candidate should be a self-driven and motivated researcher with experience in nuclear magnetic resonance techniques for determining protein structure. Prior experience in studies of protein-ligand interactions will be an added advantage. Qualified candidates should send their curriculum vitae, including contact information for three references, to **e-mail: jstreiff@uab.edu** or to: **John H. Streiff, Ph.D., The University of Alabama at Birmingham, Department of Anesthesiology, BMR2-326, 901 19th Street South, Birmingham, AL 35294.**

TEXAS A&M UNIVERSITY
Department of Biochemistry and Biophysics

The Department of Biochemistry and Biophysics at Texas A&M University **(website: http://biochemistry.tamu.edu)** invites applications for a tenure-track faculty position in the area of biochemistry. Applications at the **ASSISTANT PROFESSOR** level are particularly sought. In addition to establishing a vigorous independent research program, the successful candidate will teach at the undergraduate and graduate levels. Candidates should submit curriculum vitae, up to three reprints, a description of research plans of three pages or less, and arrange for three reference letters. All documents can be submitted electronically to **e-mail: tamu@bichsearch.tamu.edu,** or in paper form to:

Biochemistry Search Committee
Texas A&M University
Department of Biochemistry and Biophysics
2128 TAMU
College Station, TX 77843-2128

Review of applications will begin November 15, 2006, and will continue until the position is filled. *Texas A&M University is an Equal Opportunity/Affirmative Action Employer that is committed to improving diversity.*

POSITIONS OPEN

GENETICS FACULTY POSITION
Butler University

The Department of Biological Sciences invites applications for a tenure-track **ASSISTANT PROFESSOR** position with a specialty in genetics beginning in August 2007. Teaching responsibilities will include courses in general genetics, introductory biology, opportunities for upper division genetics electives, and senior seminars in the candidate's area of specialization. Experience with inquiry-based learning and/or bioinformatics is a plus. Applicants should have a Ph.D., teaching experience, and a strong commitment to undergraduate education. Successful candidates will be expected to sustain a research program involving undergraduates in genetics research. Butler has an established undergraduate research program supported by institutional funds. Set-up funds are available for the position.

We are searching for an individual to balance the teaching and research areas of the present Faculty. Applicants should submit a cover letter, curriculum vitae, a statement of teaching interests and philosophy, a statement of current and planned research, unofficial transcripts, and e-mail addresses and telephone numbers of three individuals who agree to provide letters of recommendation to: **Dr. Carmen Salsbury, Genetics Search, Department of Biological Sciences, Butler University, 4600 Sunset Avenue, Indianapolis, IN 46208.** Inquiries may be made by **e-mail: csalsbur@butler.edu.** Evaluation of applicant credentials will begin immediately upon receipt and preference will be given to those received by October 27, 2006. *Butler University is an Equal Opportunity Employer.*

NORTHWESTERN UNIVERSITY
Faculty Positions, Open Rank

The Department of Biochemistry, Molecular Biology, and Cell Biology seeks two outstanding individuals at any rank whose research programs complement the existing interdisciplinary strengths of the Department.

The Department is a vibrant and exciting research and training environment located on the undergraduate campus in Evanston, Illinois **(website: http://www.biochem.northwestern.edu).** The recent opening of the Pancoe/Evanston Northwestern Healthcare Life Sciences Pavilion is part of an ongoing expansion of the life sciences at Northwestern. Applicants should submit a cover letter, curriculum vitae, research summary, statement of future research goals, and statement of teaching experience and interests electronically as a single Adobe PDF or Microsoft Word file to **e-mail: bmbcb@northwestern.edu** using BMBCB Search as the subject. Applicants should also arrange for four letters of recommendation to be sent on their behalf. Review of completed applications will begin on November 1, 2006. To ensure full consideration, all material should be submitted by that date.

Northwestern University is an Affirmative Action/Equal Opportunity Employer. Women and minorities are especially encouraged to apply.

The Department of Biochemistry, Duke University Medical Center, invites applications for **TWO FACULTY POSITIONS** at any level. We welcome candidates in all areas of biochemistry and biomolecular sciences, particularly in structural biology, membrane biochemistry, enzymology, and nucleic acids biology. Duke is undergoing considerable expansion of its efforts in these and related areas with the recent creation of the Institute for Biological Structure and Design, which is closely affiliated with the Department. The candidates will be expected to establish strong, independent research programs and to participate in departmental teaching. Send resume, summary of research interest, and three letters of reference to: **Christian R. H. Raetz, Department of Biochemistry. P. O. Box 3711, Duke University Medical Center, Durham, NC 27710.** Consideration of applications will commence in late November 2006. *Duke University is an Equal Opportunity/Affirmative Action Employer.*

ASSISTANT PROFESSOR, BIOLOGY
Whitman College

Anticipated tenure track position in biology at the rank of assistant professor, beginning in August, 2007. Ph.D. required; post-doctoral research and/or prior teaching experience preferred. Duties will include one section of the department's required introductory Biological Principles course (with lab), and two advanced courses with laboratories. An active research program involving undergraduate students is also expected. We seek applicants in an integrative area of interest complementary to others in the department. Examples include, but are not restricted to: plant pathology; eco-physiology; plant physiology; evolutionary-developmental biology; immunology; cytogenetics; or the use of eukaryotic model systems to investigate questions in physiology or cell biology. Departmental information is available at **http://www.whitman.edu/biology**. Whitman College wishes to reinforce its commitment to enhance diversity, broadly defined, recognizing that to provide a diverse learning environment is to prepare students for personal and professional success in an increasingly multicultural and global society. In their application, candidates should address how they can contribute to diversity, a core value of the Whitman College community. Candidates should also discuss their interest in working with undergraduates as teachers and scholars in a liberal arts environment that emphasizes close student-faculty interaction. Additionally, candidates should demonstrate an interest in participation in the College's general education offerings.

Hard copy materials should include a letter of application; curriculum vitae; three letters of reference; copies of undergraduate and graduate transcripts; teaching evaluations or other evidence of demonstrated or potential excellence in undergraduate instruction; and separate statements on the candidate's teaching interests, research agenda, and potential contribution to the diversity of the College. Send to: **Integrative Biology Search, Biology Department, Whitman College, 345 Boyer Ave, Walla Walla, WA, 99362**. All materials must be received by **October 23, 2006**. Whitman College is a top-tier undergraduate institution located in historic Walla Walla, near the Blue Mountains in eastern Washington. For additional information about Whitman College, see **www.whitman.edu**.

No applicant will be discriminated against on the basis of race, national or ethnic origin, age, gender, sexual orientation, marital status, religion, or disability.

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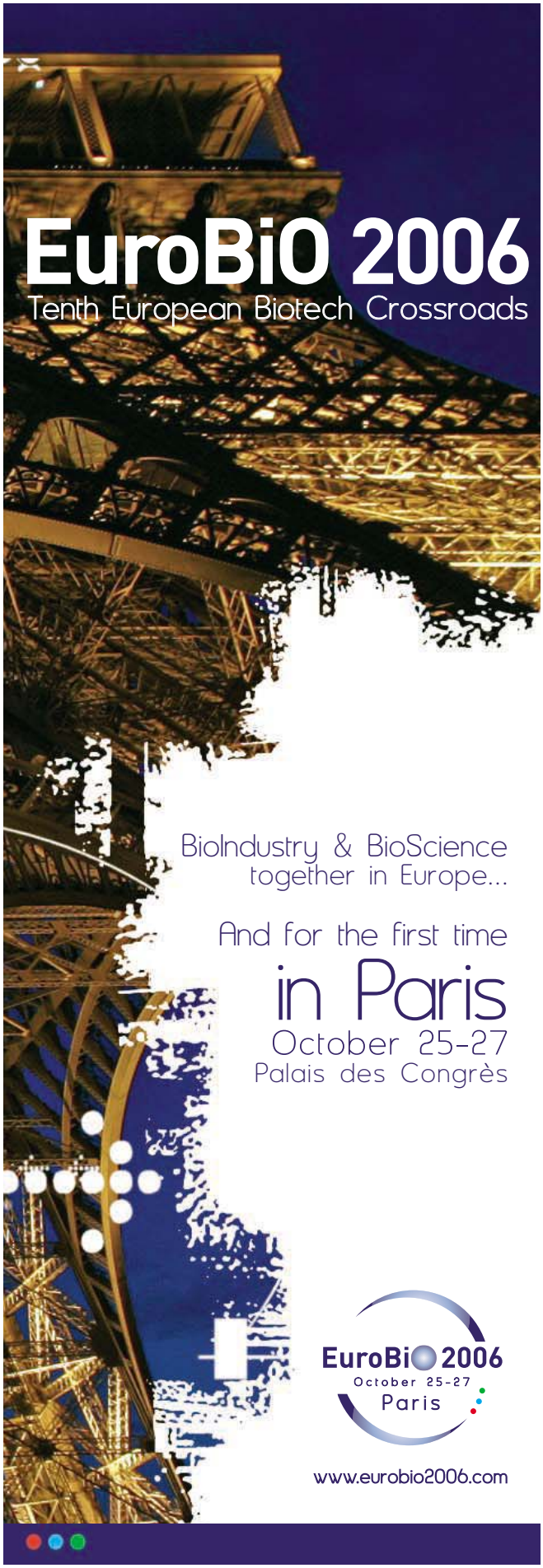

POSITIONS OPEN

ECOLOGIST

The Biology Department of Franklin and Marshall College invites applications for a one-year **VISITING ASSISTANT PROFESSOR** position in ecology, beginning July 2007 (pending administrative approval). Candidates should have the Ph.D., demonstrated strengths in teaching and research in field and laboratory settings, and broad interests in ecology and evolution. Teaching responsibilities will include lectures and laboratories in an evolution-centered introductory course that includes Mendelian genetics and ecology, and an upper-level lecture/laboratory course in ecology that emphasizes communities and ecosystems. Franklin and Marshall is a small (enrollment 1,900), highly selective coeducational liberal arts college with a tradition of excellence in science and student research and a new life sciences building to be opened summer 2007.

The successful candidate will have the opportunity to participate in our interdisciplinary major programs, including Environmental Studies/Science and Biological Foundations of Behavior (Neuroscience and Animal Behavior). Applicants should arrange to have letters sent from three referees, and should submit curriculum vitae, plans for actively engaging undergraduates through teaching and research, and undergraduate and graduate transcripts. Electronic applications will not be accepted. Priority will be given to completed applications received by November 3, 2006. Send applications to: **Dr. Mark Olson, Department of Biology, Franklin and Marshall College, P.O. Box 3003, Lancaster, PA 17604. Telephone: 717-291-4118; fax: 717-358-4548; e-mail:cindy.mcintyre@fandm.edu; website: http://www.fandm.edu/biology.xml.** *Franklin and Marshall College is a highly selective liberal arts college with a demonstrated commitment to cultural pluralism. Equal Opportunity Employer.*

ASSISTANT/ASSOCIATE PROFESSOR
Department of Biochemistry and Molecular Biology
State University of New York
Upstate Medical University

We seek applications to fill two tenure-track positions at either the **ASSISTANT** or **ASSOCIATE PROFESSOR** levels from individuals studying fundamental molecular processes in eukaryotic organisms. We encourage applications that expand existing programs, including structural biology, genomics, proteomics, and computational biology. The successful applicants will be expected to develop well-funded research programs and to contribute to medical and graduate teaching. We offer a highly competitive start-up package and salary. Further information about the Department can be found at **website: http://www.upstate.edu/biochem.**

Candidates should have a Ph.D. or equivalent, postdoctoral experience, and a strong publication record. Applicants should e-mail a PDF file containing curriculum vitae, a summary of research accomplishments and future research plans to **e-mail: biochem@upstate.edu.** In addition, three letters of reference should be mailed directly to: **Dr. Barry E. Knox, Search Committee Chair, Department of Biochemistry and Molecular Biology, 750 East Adams Street, Syracuse, NY 13210.**

Review of applications will begin on November 1, 2006, and continue until the positions are filled. *Women and minorities are highly encouraged to apply. Upstate Medical University is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL FELLOW to work on neural stem cells derived from human embryonic stem cells. Must have molecular biology background with skill in cloning and engineering genes. The ultimate goal is to design neural stem cells that can be used for transplantation.

Please send curriculum vitae and two letters of recommendation online to **e-mail: lweiner@usc.edu.**

Leslie P. Weiner, M.D., Department of Neurology, Keck School of Medicine of the University of Southern California.

POSITIONS OPEN

Washington University in St. Louis
SCHOOL OF MEDICINE

FACULTY POSITION IN DEPARTMENT OF CELL BIOLOGY AND PHYSIOLOGY
Molecular Oncology Program

The Department of Cell Biology and Physiology at Washington University School of Medicine invites applications for a tenure-track appointment at the rank of **ASSISTANT PROFESSOR.** The successful candidate will join the Molecular Oncology Program, a joint program between the Departments of Cell Biology and Internal Medicine at Washington University School of Medicine. The Molecular Oncology program is comprised of a vibrant group of interactive investigators studying cell cycle control, checkpoint control, cell death, G-protein signaling, telomere biology, HIV pathogenesis, metastasis, oncogenes, and tumor suppressors. Outstanding individuals investigating fundamental problems in molecular oncology are encouraged to apply. Candidates must demonstrate the ability to develop an independent research program and a commitment to excellence in graduate education.

Applicants must have a Ph.D. and/or M.D. and postdoctoral experience. Please send curriculum vitae, a summary of current and proposed research programs, and arrange for three letters of recommendation to be sent to:

Dr. Helen Piwnica-Worms, Chair
Cell Biology and Physiology Search Committee
Washington University
School of Medicine
660 South Euclid Avenue - Campus Box 8228
St. Louis, MO 63110
e-mail: facultysearch@cellbiology.wustl.edu

Applications should be received by March 1, 2007. *Washington University is committed to increasing representation of women and members of minority groups on its faculty and particularly encourages applications from such candidates.*

POSTDOCTORAL ASSOCIATE
in Structural Biology/X-Ray Crystallography

A senior Postdoctoral Associate position is available in the Department of Molecular Physiology and Biophysics, Mount Sinai School of Medicine to study molecular mechanisms of chromatin-mediated gene transcription. Projects involve biochemical and atomic structural characterization of macromolecular complexes by using X-ray crystallography. The successful candidate must be highly motivated and interested in pursuing independent academic research with a Ph.D. degree and demonstrated experience in protein X-ray crystallography, biochemistry, and molecular biology. Strong communication skills are desirable. Applicants should send curriculum vitae, a brief statement of research accomplishments and interests, and names of three references to: **Ming-Ming Zhou, Ph.D., Department of Molecular Physiology and Biophysics, Mount Sinai School of Medicine, New York University, P.O. Box 1677, 1425 Madison Avenue, New York, NY 10029, U.S.A., e-mail: ming-ming.zhou@mssm.edu.** *We are an Equal Opportunity Employer fostering diversity in the workplace.*

ION CHANNEL ELECTROPHYSIOLOGIST

Position available at Yale Medical School for Electrophysiologist to join multidisciplinary research group studying physiology and pathophysiology of ion channels, with emphasis on voltage-gated sodium channels. M.D. and/or Ph.D. degree and significant experience in patch clamp physiology, channel biophysics, and publications are essential. Prior work with sodium channels is desirable but not necessary. Superb opportunity to collaborate with Molecular and Cell Biologists, Molecular Geneticists, et cetera. Send curriculum vitae, statement of interest, and three letters of reference to: **Stephen G. Waxman, M.D., Ph.D., Chair, Department of Neurology, LCI 708, Yale University School of Medicine, P.O. Box 208018, New Haven, CT 06520-8018.** *Yale University is an Affirmative Action and Equal Opportunity Employer. Women and members of underrepresented minority groups are encouraged to apply.*

POSITIONS OPEN

MOLECULAR BIOLOGIST
DEVELOPMENTAL BIOLOGIST

The Biology Department at the University of Wisconsin, Eau Claire, a comprehensive liberal arts-based university with national acclaim for its excellence in faculty-undergraduate research, seeks two tenure-track **ASSISTANT PROFESSORS** in the areas of molecular biology and developmental biology. We invite applicants who share the Program's commitment to excellence in undergraduate instruction and will maintain an active research program with undergraduates. Successful candidates will possess a Ph.D. by August 20, 2007, have the potential to become excellent teachers, and have research interests that complement existing faculty. Complete position descriptions and application information are available at **website:http//:www.uwec.edu/biology.** Please send applications to the: **Department of Biology, University of Wisconsin-Eau Claire, Eau Claire, WI 54702-4004. E-mail:bogstafg@uwec.edu. Telephone: 715-836-4166. Fax: 715-836-5089.** Priority deadline November 3, 2006. *Equal Opportunity/Affirmative Action Employer.*

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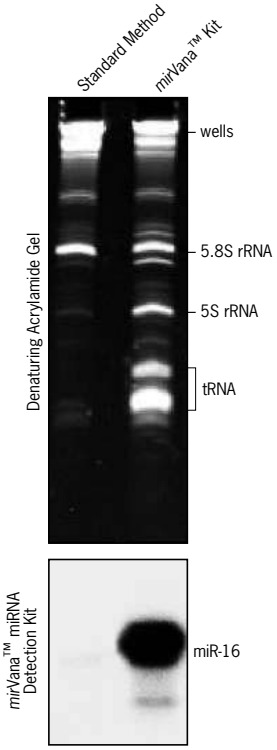

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